SYMPATHETIC NEUROEFFECTOR MECHANISMS IN ACUTELY ISCHAEMIC MYOCARDIUM

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dedicated to Susan and Katriana

DECLARATION

I declare that the work for this thesis was undertaken by me during tenure of a lectureship in the Cardiovascular Research Unit, Department of Medicine, University of Edinburgh and written up thereafter. I was the principal contributor to all sections except as indicated.

J Colin Forfar

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" Every passion of the mind which troubles mens spirit, either with grief, joy, hope or anxiety and gets access to the heart, there makes it to change from its natural constitution by distemperament, pulsation and the rest..."

Harvey W, 1628

Exercitatio Anatomic de Motu Cordis et Sanguinis in Animalibus; Francofurti Edition 1628. (Keynes English Translation, 1928).

HYPOTHESIS

"Direct liberation of catecholamines from acutely ischaemic myocardium acts as a trigger to promote early ventricular arrhythmias. Altered neurosympathetic activity may be observed by comparison of local arteriovenous catecholamine differences across ischaemic and non-ischaemic areas of the open-chest anaesthetised dog model during coronary artery occlusion under conditions of varying sympathetic tone. Interaction between the sympathetic nerve terminal, metabolic disturbances and adrenoceptor activity is likely in determining the arrhythmogenic action of catecholamine release during acute ischaemia. This model may provide increased understanding of the mechanisms of sudden cardiac death in man".

ABBREVIATIONS

[NA]	noradrenaline
[A]	adrenaline
[DA]	dopamine
LAD	left anterior descending coronary artery
[LV]	local epicardial coronary vein
[CS]	coronary sinus
I	ischaemic area of myocardium
NI	non-ischaemic area of myocardium
RMBF	regional myocardial blood flow

CONTENTS

ABSTRACT

Clinical and experimental evidence implicates the sympathetic nervous system as a possible trigger for lethal ventricular arrhythmias in ischaemic myocardium. Short term coronary occlusion in the open chest anaesthetised dog has been used as a model to study the activity of the sympathetic neuroeffector junction and its relationship to early ventricular arrhythmias. Regional sampling of coronary venous effluent on a minute-to-minute basis, estimation of myocardial blood flow and documentation of arrhythmias and epicardial activation abnormalities have been correlated with stimulation and pharmacological manipulation of the sympathetic nerve terminal to provide a combined biochemical, neuropharmacological and electrophysiological approach.

Spontaneous release of noradrenaline [NA] from ischaemic myocardium [I] and non-ischaemic myocardium [NI] is not observed during a ten minute period of regional ischaemia but does occur from [I] during early coronary reperfusion. Spontaneous ventricular fibrillation produces a secondary rise in systemic and myocardial catecholamines, usually a consequence rather than a cause of this arrhythmia. Adrenaline [A] extraction across [I] is enhanced during coronary occlusion and is abolished during early reperfusion. [NA] release from [I] during graded stimulation of the left stellate ganglion is maintained during the two early phases of enhanced vulnerability to arrhythmias but is selectively inhibited from [I] thereafter. Detrimental effects of sympathetic stimulation on epicardial activation abnormalities are seen only at the times of enhanced arrhythmia vulnerability.

Inhibition of neuronal re-uptake (desmethylimipramine) and attenuation of pre-synaptic alpha-adrenoceptors (yohimbine) potentiates nerve-stimulated [NA] release from the heart during regional ischaemia and is arrhythmogenic, intensifying activation abnormalities and further reducing blood flow to [I]. Spontaneous [NA] release from the heart during regional ischaemia is, however, only seen with these agents in combination. Both mechanisms for atten uation of sympathetic activation appear enhanced in [I] although the ability of re-uptake blocking drugs to inhibit enhanced 1-H^3-NA extraction across [I] is impaired.

Regional intracoronary potassium infusions exert biphasic effects on nerve-stimulated release of [NA] from the heart, being inhibitory at low concentration and stimulatory at high concentration with an increase in coronary vascular resistance. Both actions may coexist during regional ischaemia. Intracoronary adenosine infusions potentiate basal [NA] release from the heart but inhibit release during maximal sympathetic stimulation. Coronary vascular resistance during sympathetic stimulation is unchanged by adenosine despite major reduction in basal coronary vascular tone.

The beneficial actions of pindolol on arrhythmias, epicardial activation abnormalities, regional myocardial blood flow distribution and lactate release from [I] are principally secondary to reduction in heart rate and are substantially abolished by atrial pacing. This drug, however, reduces blood flow variance

within [I] and peak [NA] release from [NI] during sympathetic stimulation.

A potential source of error in the use of radioligands to quantify myocardial beta-adrenoceptors in crude membrane preparations is presented. False positive co-operative interactions may be suggested during I^{125} iodohydroxybenzylpindolol binding at low concentrations, due to interference by endogenous catecholamines. Preliminary studies using radioligand binding to the heart in vivo demonstrates the feasibility of this technique and changes in adrenoceptor binding during ischaemia. Non-specific binding limits the sensitivity of this approach.

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1. INTRODUCTION AND LITERATURE REVIEW

SUDDEN CARDIAC DEATH: THE PROBLEM

Sudden cardiac death, occuring within the first few minutes of symptoms of acute myocardial ischaemia or infarction as a result of coronary heart disease, remains the major therapeutic enigma in modern medicine. Between 35 and 64 years of age, one death in three results from the consequences of coronary heart disease (Gordon and Kannel, 1971) and in North America some 350,000 sudden cardiac deaths occur annually, accounting for over 50 per cent of total coronary mortality (Goldstein, 1974). The problem is particularly prevalent in Scotland where almost 40% of all male deaths in the 35-64 age group are secondary to ischaemic heart disease (Fulton et al, 1978). Despite the declining trend in cardiovascular mortality from the mid 1970's, particularly in North America, (Thorn and Kannel, 1981), sudden coronary death is likely to remain for the foreseeable future one of the most important causes of premature death in the Western World (Epstein and Pisa, 1979).

Epidemiological data are of value in the identification of subjects at risk from coronary heart disease but risk profiles based on conventional indices (cigarette consumption, lipids, blood pressure, family history, body weight, electrocardiogram) have consistently failed to predict sudden death compared to other clinical manifestations of the disease (Vedin et al, 1973; Kannel et al, 1975; Doyle et al, 1976). It is likely that sudden death from coronary disease is related more directly to pathological and metabolic changes in the heart and their consequences, than to the factors predisposing to these changes.

Mechanisms

The great majority of sudden deaths result from ventricular fibrillation, a mechanism first suggested by MacWilliam as early as 1889 and documented electrocardiographically 25 years later (Halsey, 1915). Holter monitoring during sudden cardiac death has now confirmed that ventricular fibrillation is the principal arrhythmia in 80 per cent of patients, usually preceded by varying periods of ventricular tachycardia (Nikolic et al, 1982). Atypical ventricular tachycardia (Torsade de Pointes) with an increase in QT interval immediately prior to the arrest has been documented in up to 40 per cent of cases (Panidis and Morganroth, 1982; Pratt et al, 1982). Intensive study of the mechanisms of this arrhythmia strongly supports a central role for single or multiple re-entry circuits within the heart as the basic electrophysiological process involved. The concept of circulating excitation in the heart is not new (Mines, 1913) and has been discussed throughout most of this century as a possible mechanism for ventricular fibrillation (Garrey, 1914; Lewis, 1920). In circus movement re-entry, the initiating impulse encounters an area of unidirectional block and propagates round the area of block via alternative pathways. If, as typically occurs during acute myocardial ischaemia, conduction is slow enough, tissue in the area of block can recover its excitability and the impulse can propagate retrogradely through this area emerging as a re-entrant wavefront at its site of origin. The likelihood of re-entry is therefore enhanced by a lengthy re-entry pathway, slow conduction velocity and by shortening of refractoriness. Inhomogeneities in refractoriness between adjacent cells may create focal areas of undirectional block and further potentiate the process. Several studies have shown fragmentation

of extracellular electrograms during acute and chronic ischaemia, with diastolic bridging between successively conducted beats (Durrer et al, 1961; Waldo and Kaiser, 1973; Scherlag et al, 1974; El-Sherif et al, 1975, 1977). This favours re-entrant activity in the genesis of arrhythmias and highlights heterogeneous activation patterns in the myocardium during ischaemia.

Recent experimental work has mapped circus movement re-entry during ventricular tachycardia showing the development of multiple, often incomplete, circuits of smaller diameter (approximately 0.5 cm) during ventricular fibrillation (Janse et al, 1980). A similar pattern has been documented in patients with ventricular tachycardia (Josephson et al, 1978). Such patterns have not been observed during sinus rhythm or after single ectopic beats. Injury currents at the border zone between ischaemic and normal tissue may generate ectopic activity from the non-ischaemic border zone which may act as the initiator of the re-entrant impulse (Janse and Kleber, 1981).

Ventricular fibrillation thus results from chaotic fractionated activity of the heart. Intracellular recordings have shed little light on mechanisms of pathogenesis partly because asynchrony of activity is only demonstrable in fibres separated by 10 mm or more (Hogancamp et al, 1959). A critical mass of myocardium is a prerequisite for sustained fibrillation. Atrial fibrillation, for example, is not maintained in less than one gram of tissue (Moe and Abildskov, 1959).

Anatomical substrates

It is probable that most sudden coronary deaths occur in the setting of transient myocardial ischaemia rather than evolving infarction. Thus, the Seattle experience documented acute transmural myocardial infarction (the development of new Q waves) in under 20 per cent of patients during hospitalisation following resuscitation from ventricular fibrillation (Cobb et al, 1980). Combined data from three counties in Ohio and Michigan revealed a higher incidence of infarction in such patients (44 per cent) although still only in the minority (Goldstein et al, 1981). Post-mortem studies have shown the high prevalence of severe coronary obstruction of major vessels (Perper et al, 1975; Reichenback, 1977). Coronary angiography in survivors of ventricular fibrillation confirms the high prevalence of major occlusive disease. None of the coronary arterial systems were affected more frequently than the others, with 56 per cent of 29 Seattle survivors showing greater than 70 per cent stenoses in the left arterior descending, 61 per cent in the circumflex and 41 per cent in the right coronary arteries (Cobb et al, 1975). One, two and three vessel disease was fairly equally distributed among this group. In a small subgroup of patients with a second episode of ventricular fibrillation or sudden death, angiography revealed more extensive coronary disease and increased left ventricular wall motion abnormalities (Weaver et al, 1976). Most patients dying suddenly have evidence of a previous episode of myocardial damage (gross or microscopic), often without prior history of cardiac disease (Newman et al, 1982). Many morphological studies suggest that few acute lesions are present in either the myocardium or the coronary tree (Spain and Brades, 1970; Lie and Titus, 1975;

Schwartz and Gerritz, 1975; Reichenback et al, 1977). Antemortem coronary thrombosis is rarely found; no distinctive pattern of coronary artery involvement has been identified. Although often depressed, left ventricular ejection fraction may be maintained in survivors of sudden cardiac death. Using radionuclide angiography, Ritchie et al (1982) found an ejection fraction of greater than fifty per cent in one third of 153 survivors of out of hospital ventricular fibrillation. The mean ejection fraction was 41 per cent, falling to 38 per cent during exercise. Patients resuscitated from ventricular fibrillation secondary to myocardial ischaemia are predisposed to recurrence of the arrhythmia with a 26 per cent mortality at a year and 36 per cent mortality at two years. The median time for recurrence of the arrhythmia from the Seattle study was only 20 weeks (Schaffer and Cobb, 1975). It is also clear that out-of-hospital ventricular fibrillation without infarction carries a significantly worse prognosis than that following infarction with a mortality rate three times greater at two years (Cobb et al, 1975). The poorer prognosis of survivors of out-of-hospital cardiac arrest compared to survivors of myocardial infarction has been confirmed by others (Eisenberg et al, 1982).

Prediction

If premonitory symptoms of impending ventricular fibrillation could be identified, sudden death might be avoided by rapid transportation of the patient to resuscitation facilities, preferably a coronary care unit. In two studies up to 40 per cent of patients had sought medical attention in the weeks prior to their death, but their symptoms were too vague to have been

reasonably construed as coronary heart disease (Fulton et al, 1972; Kuller et al, 1972). The Framingham and Albany studies showed that few patients reported prodromata in the days and hours prior to their death (Doyle et al, 1976).

Chronic markers of risk for sudden cardiac death have proved similarly unreliable. The mere presence of intermittent ventricular extrasystoles during a one hour period of monitoring did not increase the likelihood of sudden cardiac death over a period of three years in 1739 patients with prior myocardial infarction ;however advanced grades of ectopy increased the incidence of sudden death three fold (Ruberman et al, 1976). Although advanced grades of ventricular ectopy tend to occur in patients with impaired ventricular function, it is the former rather than the latter that is the main determinant of the probability of sudden death in this group (Schulze et al, 1977). Holter monitoring in survivors of cardiac arrest has confirmed the high prevalence of ventricular ectopy with two thirds of 144 patients showing complex patterns including bigeminy, trigeminy, repetitive or multiform ventricular activity. Over half of this group died from a subsequent arrest (Weaver et al, 1982). Unfortunately, however, this type of data comes from known high risk subgroups who form a small proportion of the population as a whole. Multiple myocardial infarctions, poor left ventricular function, cardiomegaly and heart failure are all adverse prognostic factors for cardiovascular mortality but it is not clear that death, when it comes, is more likely to be sudden in those with overt myocardial dysfunction (Gordon and Kannel, 1971). Indeed, the proportion of coronary deaths that are sudden in high risk groups is approximately 50 per cent, similar to that for the

general population (Kannel and Thomas, 1982). In almost 25 per cent of patients, sudden death is the first clinical manifestation of the disease (Lown, 1979). Recent aggressive approaches towards the control of observed and provokable arrhythmias may reduce the risks of sudden death in selected subgroups (Horowitz et al, 1980; Ruskin et al, 1981; Graboys et al, 1982), but until the arrhythmia can be accurately predicted, such measures are unlikely to have a major impact on the clinical problem as a whole. Clearly, a better understanding of the mechanisms triggering the arrhythmia is essential before rational preventative measures can be applied.

SYMPATHETIC NEURAL INFLUENCES IN NON-ISCHAEMIC MYOCARDIUM: DETERMINANT OF ELECTRICAL STABILITY

The hypothesis that neural factors, initiated locally, reflexly or from direct activation of higher brain centres, may trigger electrical instability and predispose the heart to ventricular arrhythmias is supported by considerable clinical and experimental evidence.

Sympathetic preganglionic fibres usually leave the spinal cord via the second, third and fourth thoracic anterior roots and pass from the stellate and caudal cervical ganglia to the heart (Randall et al, 1957). Anatomically, many similarities exist between the sympathetic innervation to the heart in man and that in the dog (Randall and Armour, 1977). Sympathetic fibres from the right and left side enter the heart round the adventitia of the great vessels and the atrial walls and form extensive nerve plexuses throughout the heart. Their projection corresponds to that of the main coronary arteries with distribution to surrounding tissue largely

by subepicardial routes. This has been demonstrated by surgical epicardiectomy (Szentivanyi et al, 1967) and the application of phenol to the epicardium (Randall et al, 1968), a compound producing tissue damage to a depth of approximately 0.25 mm (Kaye et al, 1968). Interestingly, vagal fibres do not appear to be disrupted by this manoeuvre (Martins and Zipes, 1980), in keeping with greater density of acetylcholinesterase staining in ventricular endocardium than epicardium (Kent et al, 1974). Adrenergic innervation is particularly rich in the sinoatrial node and to a lesser extent the atrioventricular node region. The adrenergic innervation of the ventricle is lower in density than in the atrium and tends to be less concentrated in the apical compared to basal portions of the ventricle (Randall and Armour, 1977). Although central or stellate sympathetic stimulation results in quite generalised responses across selected regions of the heart, selective stimulation of small individual cardiac nerves suggests their highly specific and localised distribution to muscle segments. In as much as each axon or nerve terminal has the capacity for independent activity, the system is potentially capable of becoming disorganised, a factor that would promote heterogeneity of myocardial conductivity, automaticity and excitability. Such heterogeneity could lead to fractionation of electrical impulses through the heart and promote arrhythmias.

Sympathetic activation:

It has been known for many years, indeed since the pioneering work by Hunt in 1899, that stimulation of the right or left stellate ganglion exerts quite different functional effects on

cardiac inotropic, chronotropic and dromotropic responses. On the right, stimulation of the recurrent cardiac nerve shortens the ventricular refractory period of the septum and to a lesser extent the anterior surface of the heart. Stimulation of the cardiac stellate nerve results in pronounced sinus tachycardia. On the left, the ventromedial and more particularly the ventrolateral cardiac nerves considerably shorten the refractory period over the posterolateral surface of the heart, mainly the left ventricle (Kralios et al, 1975). Surface ECG recordings show marked differences in repolarisation phenomena. Left stellate stimulation or right stellate ganglionectomy increases the T wave amplitude and causes prolongation of the QT interval. Right stellate stimulation or left stellate ganglionectomy increases T wave negativity with no change or a small reduction in QT interval (Rothberger and Winterberg, 1910; Yanowitz et al, 1966; Kralios et al, 1975). Inotropic responses to selective cardiac nerve stimulation show similar regional variability (Randall et al, 1968a; Armour and Randall, 1975). These studies agree with measurement of catecholamine levels in the heart following selective right or left stellectomy (Kimata, 1965). After right stellectomy, ventricular noradrenaline decreased most in the right ventricle and to a lesser extent in the anterior portion of the left ventricle. After left stellectomy, the greatest decrease in noradrenaline occurred in the posterolateral portion of the left ventricle. It is probable that vagus nerve stimulation increases refractoriness in ventricular myocardium by antagonism of sympathetic activity (Kolman et al, 1976). Electrical stimulation of vagus nerves after stellate ganglionectomy produces inconsistent effects in anaesthetised dogs (Martins and Zipes, 1980a). Studies at sites of overlapping

innervation from the right and left cardiac sympathetic nerves suggest that the left side is dominant with respect to changes in refractoriness (Haws and Burgess, 1978).

Similar differences in surface QT interval follow rapid or prolonged slow infusions of catecholamines. Brief injections of adrenaline or noradrenaline result in prolongation of the QT interval, whereas slow infusions over several minutes cause progressive shortening (Abildskov, 1976).

Since all of these interventions are known to reduce ventricular recovery time, it is likely that QT interval prolongation is the result of localised, while QT shortening is the result of more widespread influences on repolarisation. If the true time for ventricular repolarisation exceeds that recorded from a surface ECG, due to cancellation of late recovery, reduction in recovery time in a localised area of the heart could expose ECG effects in others, resulting in prolongation of the QT interval. Abildskov (1976) has provided evidence that ventricular repolarisation exceeds the duration of the QT interval by showing an increase in QT duration, exceeding the increase in QRS duration, following ectopic activation. Late periods of recovery were not evident after normal excitation. Inhomogeneities in ventricular repolarisation are a major factor influencing arrhythmia vulnerability and are increased by left stellate stimulation, digitalis glycosides, myocardial ischaemia and increase in basic cycle length (Han and Moe, 1964; Han et al, 1966). A 40 per cent reduction in ventricular fibrillation threshold follows left stellate stimulation for two minutes with reciprocal changes after ganglionectomy (Kliks et al, 1975). Spontaneous ventricular

arrhythmias (Hageman et al, 1973) and ventricular fibrillaton (Verrier et al, 1974) may follow isolated left sympathetic stimulation. In contrast, temporal dispersion of ventricular recovery is reduced by slow infusion of sympathomimetic amines (Han and Moe, 1964). This is one explanation for a possible protective effect of chronic low dose non-pressor noradrenaline infusion on ventricular fibrillation threshold (Siebens et al, 1953) and on ventricular arrhythmias and myocardial potassium loss during coronary occlusion (Regan et al, 1970). As inhomogeneities in ventricular repolarisation are a factor in both the T wave-form and arrhythmia vulnerability, analysis of the T wave might be a promising approach for determining patients at risk from arrhythmias. Body surface isopotential maps have been used in preliminary studies to represent dispersion of ventricular recovery properties with promising early results (Abildskov et al, 1977; 1978; Wyatt et al, 1978; Burgess, 1979).

Electrical stimulation of various areas of the brain can induce a variety of diverse arrhythmias even in the absence of demonstrable myocardial ischaemia (Korteweg et al, 1957; Melville et al, 1963; Abildskov et al, 1970). Changes are most marked with direct stimulation of areas with known sympathetic connections, the midbrain, posterior diencephalon and hypothalamus; stimulation of these results in repolarisation abnormalities similar to those seen with direct activation of sympathetic nerves to the heart. Combined vagal and sympathetic activation may be particularly detrimental (Manning and Cotten, 1962). Verrier et al (1975) demonstrated a 40 per cent fall in ventricular fibrillation threshold during stimulation of the posterior hypothalamus, an effect that persisted despite prevention of the associated

tachycardia and pressor response. Cervical vagotomy and bilateral adrenalectomy did not alter the fall in fibrillation threshold but it was readily prevented by beta-adrenoceptor blockade.

CNS disease:

It is well established that patients with a variety of central nervous system disease, including stroke, subarachnoid and intracerebral haemorrhage, trauma, tumours and encephalopathies may manifest striking abnormalities of ECG waveform, mainly during repolarisation and may develop ventricular arrhythmias. This association was first suggested by Byer et al in 1947 and has been widely documented (Abildskov et al, 1970; Burch, 1978). The pronounced antiarrhythmic action of sympatholytic treatment was illustrated by Grossman (1976) who reported a patient with recurrent ventricular tachycardia in association with a subarachnoid haemorrhage secondary to rupture of a basilar artery aneurysm. The arrhythmia developed with prolongation of the surface QT interval associated with tall symmetrical T waves, was resistant to lignocaine, phenytoin, procainamide, digoxin, propranolol and atropine but was immediately abolished following pharmacological blockade of the left stellate ganglion. T wave notching in patients with brain lesions may also reflect asymmetrical alterations in sympathetic tone to the ventricle (Millar and Abildskov, 1968). It is not clear whether organic cardiac lesions accompany the ECG abnormalities although petechial subendocardial haemorrhages have been reported (Koskelo et al, 1964). Myocardial lesions have also been reported following experimental intracranial haemorrhage in mice (Burch et al, 1967)

and in animals made hypertensive by stellate ganglion or midbrain reticular formation stimulation (Kaye et al, 1961; Greenhoot and Reichenbach, 1969). Some of the changes are similar to those seen experimentally after catecholamine infusion (Chappel et al, 1959; Bloom and Cancilla, 1969).

Psychological factors:

Psychological factors may play a role in cardiac arrhythmias and sudden cardiac death (Zanchetti and Malliani, 1974; Lynch et al, 1977). It is probable that the link operates through the sympathetic nervous system. At constant heart rate, the minimum current that elicited a repetitive ventricular response in dogs exposed to an aversive environment was one third that of controls (Lown et al, 1973). This increase in vulnerability was abolished following beta-adrenoceptor blockade (Matta et al, 1976).

Exercise:

Activation of sympathetic nerves to the heart through exercise can trigger arrhythmias, especially if activation is asymmetrical. Thus Schwartz and Stone (1979) noted exercise induced arrhythmias in 86 per cent of conscious dogs following right stellate ganglionectomy in contrast to 8 per cent of control animals and 11 per cent after left stellectomy. An increased incidence of arrhythmias during exercise was also documented in dogs following complete cardiac denervation with the exception of the ventrolateral cardiac nerve, a major input to the left ventricle (Randall et al, 1978). A tonic coronary vasoconstrictor influence

is present during exercise (Gwirtz and Stone, 1978; Murray and Vatner, 1978) and an increase in coronary flow following left stellectomy indicates its dependence on left sided sympathetic nerves (Feigl, 1967; Schwartz and Stone, 1977).

Long QT syndrome:

The idiopathic long QT syndrome, in addition to prolongation of the QT interval is characterised by alteration and or notching of the T wave, syncopal episodes due to ventricular fibrillation and sudden death (Schwartz et al, 1975). It was first reported by Jervell and Lange-Nielson in 1957 in association with congenital deafness but later Romano et al (1963) and Ward (1964) reported an otherwise identical syndrome occuring in patients with normal hearing. Several hundred cases have now been reported (Abildskov, 1979). Syncope usually begins in early life but may be delayed to the second or even third decade. Factors precipitating syncopal episodes include exercise, fear, anxiety and sudden noise, all of which activate sympathetic nerves to the heart. The usual mechanism of syncope is ventricular fibrillation. The most effective therapy for the syndrome involves measures that reduce neurosympathetic input to the heart such as beta-adrenoceptor blockade or left stellectomy (Schwartz et al, 1975; Malliani et al, 1980). Several findings suggest that asymmetrical sympathetic influences on the heart are involved in the pathogenesis of this unique syndrome (Crampton, 1979) although the exact nature of these influences is uncertain and could originate centrally, in the sympathetic nerves or within the myocardium. James et al (1978) reported evidence of local neuritis and neural degeneration within

the conducting tissue and ventricular muscle of eight patients who died suddenly with a history of recurrent syncope. The pattern of damage of these nerves might dictate the extent of imbalance in cardiac sympathetic activity. Kralios and Millar (1978) have shown asymmetrical maturation of cardiac sympathetic nerves in puppies which, if applicable in man, might provide a basis for regional sympathetic imbalance and subsequent arrhythmogenesis in later life. An imbalance between right and left cardiac sympathetic nerves with left dominance is supported by much experimental evidence discussed earlier.

SYMPATHETIC INFLUENCES IN ISCHAEMIC MYOCARDIUM: TRIGGER FOR EARLY ARRHYTHMIAS

Clinical Evidence:

It is now well established that most sudden deaths during acute myocardial ischaemia or infarction occur within minutes of the onset of symptoms before the patient has reached hospital (Kuller 1962; Armstrong et al, 1972; Romo, 1973). In an Edinburgh community study, 45 per cent of the total deaths within the first four weeks of myocardial infarction had occurred within the first hour of the onset of symptoms (Armstrong et al, 1972). Assessment of autonomic disturbance within this early period has shown the high prevalence of sympathetic overactivity, evidenced by tachycardia, transient hypertension or both (Webb et al, 1972). Of 240 patients, only 15 out of 89 (17 per cent) seen within 30 minutes had a normal heart rate and blood pressure when first seen. Among 151 patients first seen between 30 and 60 minutes, the incidence of autonomic disturbance was significantly lower at 56

per cent. Sympathetic overactivity was found more frequently in patients with anterior infarction and parasympathetic overactivity more frequently after posterior or inferior infarction. Sympathetic overactivity was unmasked in some patients following vagal blockade (Pantridge, 1978). Thus a temporal relationship was found between prevalence of arrhythmias and autonomic dysfunction during the critical early vulnerable period.

Although a crude guide to sympathetic nervous system activity in humans (Esler, 1982), plasma noradrenaline levels have been uniformly elevated in acute myocardial infarction (Goldstein, 1981). Most data suggests that once the infarct is established, the level of peripheral sympathoadrenal activation broadly reflects the extent of myocardial damage and its haemodynamic consequences, rather than the probability of serious early arrhythmias (Hayashi et al, 1969; Jequier and Perret, 1979; Nadeau and de Champlain, 1979; Karlsberg et al, 1981). Thus, Karlsberg et al (1981) found a four-fold rise in plasma noradrenaline and eight-fold rise in plasma adrenaline in 14 patients with acute infarction, with peripheral venous sampling a mean of two hours after the onset of symptoms. No significant differences between anterior and inferior infarction were observed and no relationship between catecholamine levels and heart rate, blood pressure or ventricular arrhythmias was evident. The close relationship between haemodynamic changes and extent of peripheral sympathetic nervous system activation has been confirmed experimentally (Karlsberg et al, 1979). Vetter et al (1974) noted plasma catecholamine levels greater than 1000 pg/ml in all of four patients sampled within one hour of the onset of symptoms. Information on cardiac sympathetic activity at the time of early enhanced vulnerability to arrhythmias was not available,

although it has been shown that the heart is capable of releasing noradrenaline later in course of infarction (Mueller et al, 1980). Earlier studies claimed higher catecholamine levels in patients with ventricular arrhythmias over the first 24 hours of infarction (McDonald et al, 1969; Griffiths and Leung, 1971), but the time scale of these measurements extended well beyond the period of enhanced vulnerability to primary ventricular fibrillation.

Increased plasma catecholamines do not necessarily imply increased spillover rates from nerve terminals. For example, elevation of plasma noradrenaline in older subjects is not due to an increase in sympathetic tone, but to diminished noradrenaline plasma clearance (Esler et al, 1981). In addition, over 80 per cent of noradrenaline released from sympathetic postganglionic neurones in man is removed by local neuronal uptake or local metabolism and does not escape into plasma (Silverberg et al, 1978).

Patients admitted to a coronary care unit with chest pain subsequently shown not to have had a myocardial infarct show lower initial levels of noradrenaline and rapid reductions towards normal during hospitalisation (Videbaek et al, 1972). Although a heterogeneous group, a number of patients in this category will have had transient myocardial ischaemia and a high risk of ventricular fibrillation. Thus it is probable that assessment of peripheral catecholamine concentrations is insufficiently sensitive for any comment to be made on the relationship between cardiac sympathetic activity and early arrhythmias during myocardial ischaemia or early infarction.

Cardiac reflexes:

There is increasing evidence that excitation of afferent nerve fibres from the heart, travelling in the cardiac sympathetic nerves, may have an important role in determining reflex sympathetic activity to the heart. This is the likely explanation of tachycardia and hypertension in patients with spontaneous angina pectoris some minutes before the onset of pain (Littler et al, 1973; Maseri et al, 1978). Myocardial ischaemia excites both vagal and sympathetic fibres from the heart and elicits a variety of reflex responses. Brown first reported in 1968 that occlusion of the left main coronary artery in lightly anaesthetised cats produced a pseudoaffective response characterised by piloerection, pupillary dilatation, limb movements, tachycardia and blood pressure changes. The response was abolished by section of cardiac afferent sympathetic fibres and was greatly reduced by section of pericoronary nerves. The cardiac sympathetic sensory endings are mechanoreceptors (Malliani et al, 1973; Casati et al, 1979), but their activity can be further enhanced by chemical substances such as bradykinin (Lombardi et al, 1981) which are released from the ischaemic heart.

Activation of the afferent limb is followed by increased efferent discharge of cardiac sympathetics, an excitatory cardiocardiac reflex (Malliani et al, 1969). This reflex can be activated within seconds of the onset of myocardial ischaemia and may play an important role in arrhythmogenesis at this time, since interruption of the afferent limb by section of the dorsal roots from the eighth cervical to fifth thoracic segment can substantially reduce arrhythmias associated with short-lived

coronary occlusion (Schwartz et al, 1976). It has been suggested in a preliminary study that efferent neural activation of cardiac sympathetic nerves after coronary occlusion is patchy, being increased in some but decreased in others (Lathers et al, 1976). Selective activation of individual nerves to the heart is a well recognised arrhythmogenic manoeuvre (D'Agrosa and Flannigan, 1976). Cardiac sympathetic afferents are also capable of inhibiting cardiac efferent vagal discharge (Schwartz et al, 1973) and may thus antagonise vagally-mediated increases in cardiac electrical stability (Kolman et al, 1975; De Silva et al, 1978). It might be expected that activation of efferent cardiac sympathetic discharge following coronary occlusion would be demonstrable in non-ischaemic as well as ischaemic areas of the heart. Pashkow et al (1977) have shown increases in myocardial contractile force in non-ischaemic myocardium following coronary ligation for two minutes, a response abolished by transection and cannulation of the artery supplying the non-ischaemic region.

Cardiac sympathetic afferent nerves have also been shown to have excitatory influences on renal sympathetic nerve activity during acute ischaemia (Weaver et al, 1981), such a reflex possibly contributing to inappropriate hypertension at this time. This in turn may increase myocardial oxygen demands, intensify the area of ischaemia, and exacerbate myocardial electrical instability through a cycle of reflex neural discharge.

Cardiac denervation:

In 1936, Cox and Robertson suggested that thoracic sympathectomy reduced mortality from ventricular fibrillation

following coronary ligation although statistical evaluation of their data indicates that mortality rates between control and denervated groups were not significantly different (Corr and Gillis, 1978). Cox and Robertson are credited with first advancing the hypothesis that neural reflexes from the heart might be deleterious to its function (Malliani et al, 1980). Early studies on the effect of bilateral stellate ganglionectomy and removal of chain ganglia showed a variable and at times inconsistent protection against arrhythmias and mortality during the first few minutes of myocardial ischaemia (Harris et al, 1951; Milch et al, 1955; Schaal et al, 1969). Depletion of myocardial catecholamines with reserpine has also shown inconsistent effects on arrhythmias, including ventricular fibrillation, following coronary occlusion (Maling et al, 1959; Melville and Varma, 1962; Sommers and Jennings, 1972). One possible explanation for these inconsistences lies in the inability of these interventions to achieve complete cardiac denervation. Thus, residual islets of functioning adrenergic nerve terminals may actually exacerbate the tendency for arrhythmia development. Similarly, reserpine rarely causes complete depletion of catecholamine stores and with levels as little as five per cent of control, effective neurotransmission can take place, at least in part through postsynaptic hypersensitivity (Kalsner and Nickerson, 1969).

A second explanation for the variable effectiveness of sympatholytic interventions was suggested by Ebert et al (1970) by showing that chronic, but not acute cardiac denervation was protective against early ventricular arrhythmias. These authors suggested that adequate time must elapse to allow depletion of catecholamines from the heart before protection is evident. More

recently, Thomas et al (1981) have supported the concept of a salutory effect of chronic, but not acute cardiac denervation on cardiac muscle mechanics during ischaemia. Cardiac contractile force in the ischaemic area declined by 67 per cent in the control dogs, by 74 per cent in acutely denervated dogs but by only 22 per cent in the chronically denervated group ($p < 0.001$). This data highlights the importance of direct local interactions between ischaemic muscle and sympathetic terminals.

Current techniques for surgical denervation of the heart, mainly developed in Randall's laboratory, allow complete, verifiable, selective cardiac denervation and have been used to study possible mechanisms for the cardioprotective effects of this manoeuvre (Jones et al, 1978; Randall et al, 1980). In studies where infarct size has been determined, chronically denervated dog hearts had infarcts some 80 per cent smaller than sham-denervated controls, whereas in acutely denervated hearts, infarct size was reduced by only 25 per cent (Jones et al, 1978a). The mechanism for this protection is uncertain but may in part be related to reduction in oxygen consumption of chronically denervated heart tissue (Gregg et al, 1972). Heart rate, a major determinant of myocardial oxygen consumption, was also reduced by acute and chronic denervation but perfusion pressure tended to fall also, making the net balance between oxygen delivery and oxygen demand difficult to determine.

At similar heart rates, blood pressure and levels of cardiac work, Barber et al (1980) found significantly lower left ventricular epicardial and endocardial blood flow (measured by radiolabelled microspheres) in chronically denervated compared to

sham-denervated dog hearts. Local ST segment elevation, measured by epicardial and endocardial plunge electrodes, was less in denervated hearts in low flow areas following ligation of the left anterior descending coronary artery and arrhythmias were less frequent. Collateral perfusion to the ischaemic area was not modified by denervation in this study, but others have suggested up to a four fold increase in blood flow in central and peripheral ischaemic regions after this procedure (Jones et al, 1978b).

Sympathetic stimulation and attenuation:

Central, hypothalamic stimulation during acute myocardial ischaemia readily exacerbates the tendency for ventricular fibrillation. Lown and Verrier (1978) noted ventricular fibrillation in 63 per cent of dogs during coronary artery occlusion and hypothalamic stimulation, contrasting with a 6 per cent incidence of fibrillation without hypothalamic stimulation. Hypothalamic stimulation failed to induce arrhythmias in any animals without ischaemia.

A similar pattern of enhanced vulnerability to arrhythmias follows stellate ganglion stimulation during ischaemia, with the increased incidence of ventricular fibrillation independent of changes in heart rate and blood pressure (Verrier et al, 1974; Kliks et al, 1975). Aversive environmental conditioning may also provoke arrhythmias without direct activation of sympathetic nerves in dogs following coronary occlusion (Corbalan et al, 1974).

The asymmetrical influences of cardiac sympathetics in ischaemic myocardium are well documented. Interruption of the left

stellate ganglion attenuates the arrhythmias, whereas right stellectomy enhances the frequency of arrhythmias, including ventricular fibrillation (Schwartz et al, 1976a). Interruption of sympathetic activity at the level of the dorsal roots of the spinal cord (Schwartz et al, 1976) or interruption of spinal function from C4 to T6 (Bhargava et al, 1976) is also protective. Asymmetrical sympathetic influences may also operate in the myocardium as a result of previous infarction. Barber et al (1983) recently showed that experimental infarction can lead to sympathectomy of healthy myocardium distal to the infarct, presumably as a result of interruption of epicardial sympathetic nerves which travel beside the coronary arteries between base and apex (Randall et al, 1968).

Cardiac sympathetic tone may also influence patterns of coronary blood flow during critical coronary artery stenosis. Cyclical reductions in flow, characterised by Folts et al, 1976, and probably mediated via aggregation and disaggregation of platelets (Kowey et al, 1981; Folts et al, 1982), are enhanced by stellate ganglion stimulation and adrenaline infusion and diminished by stellectomy (Raeder et al, 1982).

DETRIMENTAL EFFECTS OF CATECHOLAMINES IN ISCHAEMIC MYOCARDIUM

Electrophysiology:

Local liberation of noradrenaline from nerve terminals in ischaemic myocardium may potentially trigger arrhythmias through a variety of different but not mutually exclusive mechanisms.

Catecholamine-enhanced diastolic depolarisation of specialised ventricular muscle fibres increases normal automaticity (Tsien,

1974) even in the presence of partial depolarisation (Noble 1975). However, diastolic depolarisation can be effectively inhibited by elevating extracellular potassium concentration to a level likely to be present in ischaemic myocardium so it is doubtful whether this mechanism operates in vivo (Imanishi and Sura$^{W}_{I}$icz, 1976).

Triggered after-depolarisations (Cranefield, 1975) may develop at several levels of transmembrane potential, and their amplitude is increased by the action of noradrenaline (Hoffman, 1978). As the amplitude of these oscillatory afterpotentials is partly a function of cycle length, catecholamines may allow them to reach threshold levels by increasing heart rate.

Possibly the most important electrophysiological action of catecholamines in acutely ischaemic myocardium lies in their ability to promote re-entrant excitation. In the presence of increased extracellular potassium, the fast inward sodium channel becomes inactivated and a calcium-dependent slow response action potential, with an extremely slow conduction time, is generated (Wit et al, 1972). This will facilitate re-entrant activity within the heart. Higher levels of potassium increase potassium conductance and may prevent propagation of the impulse. In the presence of catecholamines, however, slow response action potentials may be reactivated (Carmeliet and Vereecke, 1969), with slow propagation through the ischaemic area and completion of re-entrant circuits leading to ventricular fibrillation. Sympathetic neural stimulation may even improve conduction in ischaemic tissue (Millar et al, 1976) by allowing impulse propagation through areas previously showing conduction block. The conducting impulse may then propagate back through previously

refractory tissue and complete a re-entrant pathway.

Metabolism:

Ultimately, the arrhythmogenic consequences of local catecholamine release on ischaemic heart muscle depend on neurotransmitter actions on cellular metabolic processes (Figure 1.1).

Catecholamines stimulate adenylate cyclase activity in the myocardial cell membrane (reviewed by Drummond and Severson, 1979) by a molecular mechanism involving interaction with at least three membrane proteins: the $beta_1$-adrenoceptor, the catalytic subunit of the enzyme and a guanyl nucleotide binding regulatory component (Schultz and Jakobs, 1981). Elevation of cyclic adenosine 3', 5' monophosphate (cAMP) results and has been shown in ischaemic regions prior to malignant arrhythmias in the cat (Corr et al, 1978), the baboon and to a lesser degree the dog (Podzuweit et al, 1978). The rise in cAMP is associated with a fall in ventricular fibrillation threshold (Lubbe et al, 1978). There is considerable evidence that cAMP influences calcium channels in the sarcolemma; increased calcium entry into heart cells influences both their contractile state and electrical activity (Reuter, 1974), and is involved in the development of both reversible and irreversible cell injury (Reimer and Jennings, 1981). Although the molecular mechanisms are poorly understood (Benfey, 1980), it is generally accepted that an increase in cytoplasmic calcium concentration also represents the main intracellular signal following activation of the $alpha_1$-adrenoceptor. Rapid changes in the activity of cardiac protein kinase enzymes may follow these intracellular signals

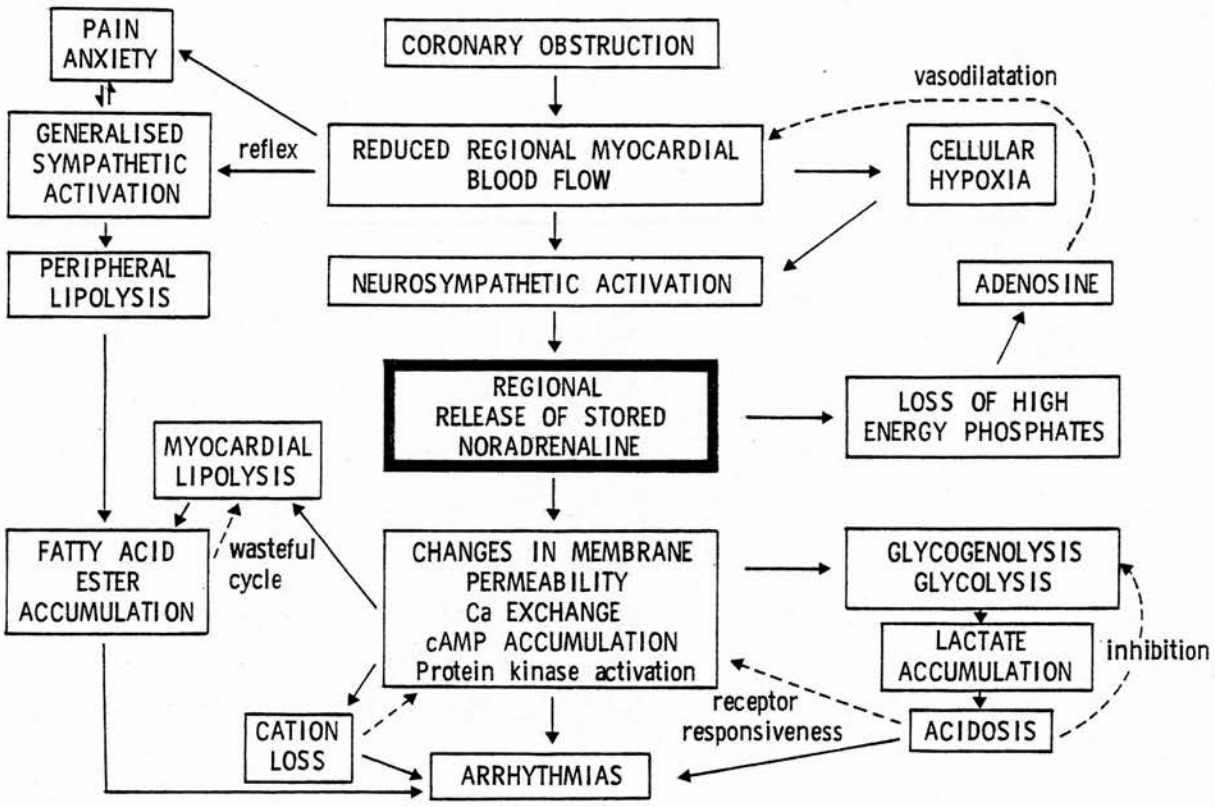


Figure 1.1 Interrelations between neurosympathetic activity and metabolism in acutely ischaemic myocardium.

(Mewes and Hofmann, 1981) and determine the energy balance of the ischaemic cell. For example, cAMP dependent activation of phosphorylase-kinase will activate glycogenolysis and provide endogenous substrate for ATP production via anaerobic glycolysis.

The increase in contractile force (in normal or peri-ischaemic muscle) and heart rate following adrenergic stimulation will increase myocardial oxygen demands and hasten the depletion of energy reserves. Progressive loss of the cellular pool of high energy phosphates will thus be accelerated and, at least in more severely ischaemic muscle, their replacement by glycolytically produced ATP will be limited because of end-product inhibition of anaerobic glycolysis by acidosis and lactate production (phosphofructokinase inhibition) and by a rise in the NADH/NAD ratio (glyceraldehyde-3-phosphate dehydrogenase inhibition) (Neely and Morgan, 1974; Rovetto et al, 1975).

A further deleterious action of catecholamines on ischaemic cell energetics operates through changes in lipid metabolism. Activation of peripheral lipolysis following acute myocardial ischaemia increases free fatty acid (FFA) concentrations (Oliver et al, 1968) and increases their uptake by the myocardium (Vik-Mo et al, 1979). The latter is principally dependent on the fatty acid-albumin molar ratio (Miller et al, 1976). This may enhance myocardial oxygen utilisation without influencing myocardial mechanics (Mjos 1971), possibly through the cycling of these acids into and out of the triglyceride pool by ischaemia-induced esterification to triglyceride and lipolytic breakdown to FFA and glycerol. The extent of elevation of plasma FFA in man following acute infarction has been linked both to infarct size and the

development of serious ventricular arrhythmias (Oliver et al, 1968; Opie et al, 1977). Reduction in FFA availability to the myocardium by increasing the albumin-FFA ratio (Miller et al, 1976) or by antilipolytic drugs (Mjos et al, 1976; Vik-Mo, 1977), decreases epicardial currents of injury. Inhibition of beta-oxidation in ischaemic cells promotes the accumulation of triglycerides (Wartmann et al, 1956; Scheuer and Brachfeld, 1966) which may increase cellular oxygen requirements and the tendency for arrhythmias (Brownsey and Brundt, 1977). Enhanced endogenous lipolysis in ischaemic myocardium is at least partly catecholamine dependent, as in rats pretreated with reserpine ischaemia failed to stimulate hormone sensitive lipase activity (Hough and Gevers, 1975). Accumulation of long chain fatty acyl CoA esters may inhibit the transport of high energy phosphates across the mitochondrial membrane (Shung et al, 1975).

PURPOSE OF THESIS

It is a reasonable hypothesis that local and particularly heterogeneous release of catecholamines in ischaemic myocardium could act as a trigger for metabolic and electrophysiological derangements promoting lethal ventricular arrhythmias. Despite broad-based evidence in support of this contention, a paucity of direct information is available concerning sympathetic nerve terminal activity in acute ischaemia and the mechanisms controlling noradrenaline release and adrenoceptor activation at this time.

This thesis examines spontaneous and nerve-stimulated overflow of sympathetic neurotransmitters in an animal model of myocardial ischaemia in vivo at the time of development of the early phase of

spontaneous ventricular arrhythmias, and relates this overflow to regional electrophysiology, haemodynamic and some metabolic abnormalities across ischaemic and non-ischaemic areas of the heart. The specifity of the model in allowing sampling of regionally ischaemic and non-ischaemic venous effluent is also critically evaluated. The role of presynaptic mechanisms controlling neurotransmitter overflow during ischaemia is assessed using alpha$_2$-adrenoceptor and neuronal reuptake blocking drugs and the actions of some ischaemic metabolites evaluated using regional infusion techniques. Prospects for the prevention of beta-adrenoceptor mediated detrimental electrophysiological effects and the role of tonic sympathetic stimulation in ischaemic tissue are evaluated by analysis of the haemodynamic and electrophysiological effects of a beta-adrenoceptor blocking drug with intrinsic sympathomimetic activity. Finally, methodical aspects of beta-adrenoceptor quantification in vitro using radioligand binding techniques are studied and their potential application to adrenoceptor quantification in vivo explored.

It is the aim of this thesis to provide increased basic knowledge of the complex interrelationships between cardiac neurosympathetic activity and metabolic and electrophysiological derangements in acutely ischaemic myocardium at the time of onset of early ventricular arrhythmias. A direct approach of assessing neurosympathetic activity, namely regional catecholamine release, has been adopted. It is hoped that the studies may provide a more rational therapeutic approach as a basis for the prevention of sudden cardiac death from coronary heart disease.

2. MATERIALS AND GENERAL METHODOLOGY

THE MODEL

Detailed clinical studies of the mechanisms of ventricular fibrillation during the first few minutes of myocardial ischaemia are impractical. The use of an open-chest anaesthetised dog model of acute myocardial ischaemia is based on the following considerations.

Firstly, it allows simulation or partial simulation of the mechanisms of pathogenesis of lethal ventricular arrhythmias in acute ischaemia that may be applicable to sudden cardiac death in man (Bigger et al, 1977). The early phase of ventricular arrhythmias in the dog following coronary artery ligation has been particularly well characterised since its description by Harris in 1950. Serious ventricular arrhythmias, including ventricular fibrillation, are common during the first 20 minutes of acute ischaemia in this model (Harris phase 1 arrhythmias). After 30 minutes, arrhythmias are rare for several hours. Two peaks of arrhythmogenesis have now been characterised during this early period, the first 5-6 minutes after coronary ligation (phase 1a) and the second 15-17 minutes after coronary ligation (phase 1b) (Haase and Schiller, 1969; Meesman et al, 1978; Kaplinsky et al, 1979). Reproducible electrophysiological, metabolic and haemodynamic changes follow short periods of ischaemia (10-15 minutes), without the development of myocardial necrosis (Braunwald and Kloner, 1982) and allow comparison of control and intervention periods (Kjekshus and Mjos, 1972; Maroko and Braunwald, 1976; Mjos et al, 1976).

Secondly, the physiology of the neurosympathetic cardiac axis has been particularly well studied in the dog (Randall, 1977) and

detailed information is available concerning regional distribution, function and neuropharmacology of cardiac sympathetic nerves in this species. Neurosympathetic activity and responsiveness has, however, been poorly characterised during the early phase of arrhythmogenesis in acute ischaemia.

Thirdly, the use of an open-chest intact animal permits integrated reflex, central and peripheral responses to acute ischaemia to take place, while allowing regional analysis of metabolic and electrophysiological variables across both ischaemic and non-ischaemic regions on a minute-to-minute basis. Dog heart shows similar metabolic and electrophysiological responses to that of primate (baboon) heart (Opie et al, 1975). Sheep heart has unusual coronary vasculature, while pig heart is characterised by lack of intercoronary anastomoses and a conduction system penetrating to epicardial layers, unlike that in man and in the dog. Regional venous sampling on a minute-to-minute basis, a technique widely used in this thesis, is technically difficult in the rather small cat heart.

The need for anaesthesia constitutes a potential disadvantage of the open-chest preparation as levels of anaesthesia may influence myocardial mechanics, oxygen consumption, metabolism and cardiovascular reflexes and hence may effect changes in ventricular vulnerability during acute ischaemia (Prys-Roberts, 1980). In all studies, care was taken to ensure an adequate and even level of anaesthesia throughout, with maintenance of normal acid-base balance and normothermia by the use of a heating blanket. Systemic hypoxia acts as a major trigger for activation of cardiac sympathetic activity (Woods and Richardson, 1959; Downing, 1966)

and was avoided by continuous positive pressure ventilation with room air using a Harvard respirator at a tidal volume and rate derived from a standard nomogram according to body weight. Neither respiratory acidosis nor respiratory alkalosis alter the ventricular fibrillation threshold in dogs (Gerst et al, 1966) although indirect effects are possible through depression of myocardial contractility.

Initial studies investigating spontaneous outflow of myocardial catecholamines in acute ischaemia used α-chloralose in urethane as the anaesthetic agent with morphine premedication. In a subsequent series, α-chloralose alone was used to avoid possible vagotonic effects of morphine (De Silva et al, 1978a) that might have inhibited nerve terminal catecholamine release (Levy, 1982). It has been claimed that α-chloralose causes less depression of cardiovascular reflexes than other intravenous anaesthetics. Tonic parasympathetic tone may be preserved to a greater degree than with other agents (Van Citters et al, 1964), a factor that can influence local electrogram abnormalities during acute ischaemia (Ruffy et al, 1981).

Pentobarbitone anaesthesia was used for all studies using sympathetic nerve stimulation and manipulation of nerve terminal neuropharmacology. It is recognised that vagal tone is low with barbiturate anaesthesia (Priano et al, 1969) and there is some evidence that sympathetic tone may be enhanced (Ruffy et al, 1981). Barbiturate anaesthesia may change ventricular refractoriness (Laforet et al, 1957), cardiovascular dynamics (Cox, 1972), and under certain circumstances may exert significant anti-arrhythmic effects (Hoffmeister et al, 1958). Dawson et al (1980) compared

indices of ventricular vulnerability (fibrillation threshold, single and multiple repetitive extrasystole threshold) in dogs with and without pentobarbitone anaesthesia. Small but significance reductions in these parameters were observed in the conscious state. Fifty-one per cent of the variability in fibrillation threshold when conscious was explicable by results during prior anaesthesia, whereas 77 per cent of the variability was explicable in a control group during two periods of anaesthesia. No detailed studies are available comparing the effects of barbiturate anaesthesia on indices of ventricular vulnerability during ischaemia. The use of each animal as its own control should minimise experimental error as a consequence of variability in anaesthetic agent or in levels of anaesthesia.

Local cooling of the heart as a consequence of air exposure following thoracotomy can alter action potential duration, refractory periods and conduction times (Hoffman, 1959). Effects on arrhythmogenesis are more marked if temperature gradients are generated across the myocardium (Wallace and Mignore, 1966) and are related to the speed of generation of such gradients. Temperature differentials may be significant between epicardium and endocardium and may even be enhanced during ischaemia when reduced coronary flow diminishes heat transfer.

Significant loss of plasma volume can follow ventilation as a result of insensible fluid loss and therefore a continuous intravenous infusion of isotonic saline was used in all experiments. Additionally, blood losses during surgery were replaced by saline.

The validity of the technique of assessment of metabolic

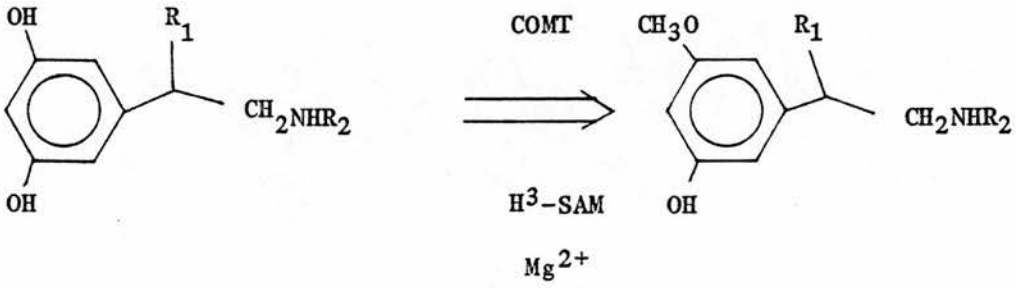
changes during myocardial ischaemia from regional arteriovenous differences requires careful consideration. Although arterial blood perfuses the ischaemic region via collaterals from non-occluded vessels, local venous effluent from the ischaemic zone represents a mixture of venous blood draining both ischaemic and non-ischaemic myocardium. Furthermore, venous effluent from ischaemic tissue will be an admixture of contributions from tissue with varying degrees of hypoperfusion, such that the greater the degree of ischaemia the less the relative contribution to venous effluent and the greater the likely dilution with venous effluent from less severely ischaemic or even non-ischaemic regions. Regional redistribution of blood following the release of vasoactive metabolites during the early minutes of ischaemia can further influence venous admixing with time, irrespective of the severity of ischaemia. However, striking metabolic changes are detectable from biochemical analysis of venous effluent draining ischaemic myocardium. Regional lactate production, potassium release and enhanced glucose extraction have all been demonstrated during ischaemia, even if the magnitude of change in coronary venous effluent in, for example, lactate and potassium is considerably less than within the central ischaemic zone. A stricter comparison of ischaemic arteriovenous versus non-ischaemic arteriovenous substrate differences is achieved by simultaneous sampling of local venous effluent draining a non-ischaemic area of myocardium. Under normal conditions, overflow of noradrenaline from the myocardium is a small fraction of the released neurotransmitter, probably less than 10 per cent, mainly due to the activity of neuronal reuptake processes. Changes in neurosympathetic activity, local blood flow, neuronal reuptake and

extraneuronal reuptake as a consequence of acute ischaemia complicate the prediction of noradrenaline output after coronary occlusion and hence the release from the heart has generally been expressed as an arteriovenous concentration difference. Detailed analysis of the validity and assumptions involved with the regional venous sampling technique is presented on pages 55-63 .

Assessment of regional myocardial blood flow using radiolabelled microspheres has the advantage of providing fairly accurate estimates of regional flow in multiple endocardial and epicardial tissue sites. General criticisms may be applied to the microsphere technique (Buckberg et al, 1971). The random distribution of spheres injected into the arterial circulation means that precision of regional flow determination is related to the number of spheres in each tissue sample. The requirement that there should be at least 400 spheres per sample dictated the total number injected. With very low flow, variability in derived flow secondary to random redistribution of small numbers of spheres would be increased. Higher doses of microspheres were used for detailed central and border zone blood flow analyses. Plasma streaming, preferential sphere distribution and failure of sphere trapping are minimised by the use of spheres 15 μ in diameter : smaller spheres have a higher incidence of escape through the vascular bed into the venous circulation and larger spheres may show preferential regional flow distribution. No haemodynamic disturbances have been reported in the literature following the injection of the doses used in this thesis.

RADIOENZYMATIC ASSAY OF PLASMA (AND TISSUE) CATECHOLAMINES

Principle: The catecholamines noradrenaline [NA], adrenaline [A] and dopamine [DA] are converted to their 3-0-methylated derivatives using catechol-0-methyl transferase (COMT) in the presence of a radioactive methyl group donor, H^3-methyl-S-adenosyl methionine.



R_1 = H; R_2 = H	Dopamine
R_1 = OH; R_2 = H	Noradrenaline
R_1 = OH; R_2 = CH_3	Adrenaline

The radioactive products normetanephrine (from [NA]), metanephrine (from [A]) and methoxytyramine (from [DA]) are purified by selective extraction and separated by thin layer chromatography (TLC). Normetanephrine and metanephrine are oxidised to vanillin.

This method is modified from Da Prada and Zurcher (1976). It has advantages of simultaneous determination of [NA], [A] and [DA], high sensitivity and specificity (within the femtomole range) and low sample volume.

Extraction of COMT: COMT was purified from rat liver adapting the procedure of Axelrod and Tomchick (1958). All procedures were performed on melting ice or at 4°C. Rat liver (100 g : 10 rats) was homogenised in 400 ml 1.19% KCl, filtered through two layers of nylon gauze, and the homogenate centrifuged for 10 minutes at 15000 rpm in a refrigerated centrifuge. The fluffy white floating layer

was discarded and the supernatant centrifuged at 24000 rpm (65,000 g) (Beckman ultracentrifuge) for 30 minutes. The pH of the supernatant was adjusted to 5.0 using 1 M acetic acid, left for 20 minutes and the precipitated proteins spun down (30 minutes, 24000 rpm) and discarded. Ammonium sulphate (16.1 g in 100 ml) was added slowly with constant stirring and precipitated proteins spun down (30 minutes, 24000 rpm) after 15 minutes and discarded. More ammonium sulphate was added (14.8 g in 100 ml) and left for 15 minutes. The precipitate from this stage, containing the COMT, was packed by centrifugation (30 minutes, 15000 rpm) and the pellet resuspended in 30 ml 1 mM sodium phosphate buffer (pH 7.0), and dialysed for 15 hours against the same buffer (51.1 mM $NaPO_4$) containing 0.1 mM dithiotreitol (DTT) using dialysis tubing (circumference 48 mm) cleaned three times in 10^{-4} M EGTA. The final product was centrifuged (10 min, 15000 rpm) and the clear supernatant aliquoted into 1 ml cups and deep frozen. The enzyme is stable for at least three months at -20°C.

Specified Reagents:

(1) H^3-methyl-S-adenosyl methionine. Radiochemical Centre, Amersham. Specific activity 10 Ci/mmole (stored -40°C).

(2) HCl Analar BDH-10125. 37% diluted DDW.

(3) Perchloric acid $HClO_4$, Analar BDH. 72% diluted DDW.

(4) Acetic acid ION, Analar BDH. diluted DDW.

(5) NaOH 5N Analar BDH. diluted DDW.

(6) Ammonia 2N Merck 25% diluted DDW.

(7) Tris buffer 2M, Aristar BDH - 45205. pH 9.6.

(8) Borate buffer 1M, Analar BDH - 10058. pH 8.0

(9) Dopamine stock solution (100 µg/ml), Dopamine HCl, Kochlight

3283.00 in 0.01 N HCl. Stored 4°C. Fresh every 2 months.

(10) Adrenaline stock solution (100 µg/ml). L-adrenaline hydrogen tartrate. Kochlight 9779-60 in 0.01 N HCl. Stored 4°C. Fresh every 2 months.

(11) Noradrenaline stock solution (100 µg/ml). L-noradrenaline bitartrate. Kochlight 4351-70 in 0.01 N HCl. Stored 4°C. Fresh every 2 months.

(12) $MgCL_2$ 500 mM. Merck. Diluted DDW.

(13) Dithiotreitol (DTT) A grade. Calbiochem.

(14) Enzyme mix:

DTT (13)	1 mg
Tris buffer (7)	500 µl
COMT	250 µl
$MgCl_2$ (12)	200 µl
SAM (1)	20 µl
	970 µl

(15) Carrier solution:

3 methoxytyramine (HCl) (Calbiochem 45426)	30.5 mg
DL metanephrine (HCl) (Calbiochem 44797)	29.6 mg
DL normetanephrine (HCl) (Calbiochem 4911)	30.0 mg
HCl 0.01 N	to 25 ml

(16) Sodium tetraphenylborate (TPB) 1.5%, Merck pH 8.0. Diluted DDW.

(17) TLC tank solvent:

Chloroform	Merck	80 ml
Ethanol	Merck	15 ml
Ethylamine	Merck	10 ml

(18) Scintillation solution:

Butyl PBD (Ciba)	12.5 g
Toluene (Merck)	2.5 l

(19) Elution solvent for methoxytyramine:

Triton-X-100	20 g
Glacial acetic acid	100 ml
Methanol	to 1 l

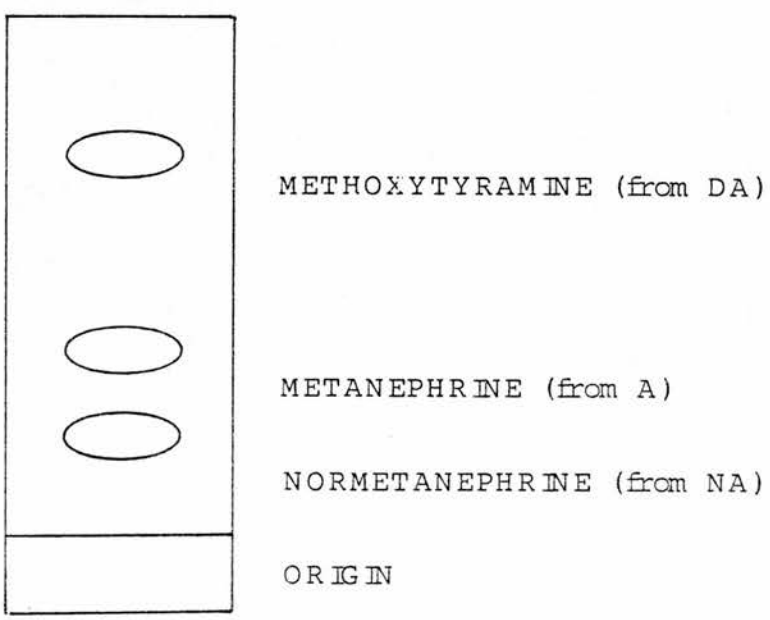
Procedure: Plasma (100 µl, frozen immediately after separation from blood collected into precooled tubes (4°C) containing 10 µ heparin and 1 mg sodium metabisulphate) was deproteinised using 1 vol 0.6 N $HClO_4$, mixed and centrifuged for 2 minutes. Oxidised plasma samples were prepared by adding 2 mg $NaIO_4$ to 1 ml plasma and oxidation at 35°C for 10 minutes before deproteinisation as described above. Tissue samples were weighed, homogenised in 10 vols 0.3 N $HClO_4$ (containing 5.6 mM EGTA), the homogenate centrifuged (10 minutes, 15000 rpm) and the supernatant decanted. The pellet was re-extracted in $HClO_4$ solution and centrifuged before combining the extracts and proceeding as for plasma.

The methylation reaction was undertaken for one hour (35°C), shaking slowly, according to the following scheme:

	SAMPLE	BLANK	OX BLANK	STAND	INT STAND
Deproteinised plasma	100	-	-	-	100
(Oxidised plasma)	-	-	100	-	-
0.3N $HClO_4$ (1 mg/ml Asc Acid)	-	100	-	100	-
Ref Stand (NA, A, DA 60,600 fmol/50 µl from stock soln.)	-	-	-	10-50	50
0.01 N HCl	50	50	50	0-40	-
Enzyme mix	100	100	100	100	100

Extraction of 3-O-methylated products: The reaction was stopped by placing tubes in an ice-cold bath and adding freshly prepared borate buffer (8), 3 parts and carrier solution (15) 1 part (200 µl). 100 µl TPB (16) was added and the mixture extracted into 10 ml diethylether by shaking for 5 minutes. After centrifugation (5 minutes, 1500 rpm) the water phase was frozen on dry ice, the ether phase added to 0.5 ml 0.1N HCl, shaken for 5 minutes and recentrifuged before freezing the water phase again on dry ice. The ether phase was discarded and the acid phase (containing the methylated products) washed with 5 ml butylacetate, shaken for 5 minutes, centrifuged and the water phase frozen. The butylacetate phase was discarded. The acid phase was evaporated to dryness under vacuum and the residue dissolved in 100 µl 0.1N HCl.

Amine separation: 50 µl of purified extract was carefully spotted onto precut 3 x 20 cm, TLC plates (LK5E, Whatman) with a preadsorbed origin, equilibrated with 105 ml solvent solution and chromatographed for 1 hour in a solvent tank (approx. 12 plates per tank). The TLC plates were dried in a stream of cold air and the individual catecholamines identified under ultraviolet light.



Methoxytyramine (Rf value 0.7) was not assayed in the studies described but the method allows this peak to be removed, eluted and counted directly. Metanephrine (Rf value 0.5) and normetanephrine (Rf value 0.3) were scraped individually and transferred to liquid scintillation vials. 1 ml 2N ammonia (6) was added, shaking for 5 minutes and the oxidation stopped by the addition of 50 µl 10% glycerol solution. The pH was adjusted by the addition of 0.5 ml 10N acetic acid (4) and 10 ml scintillation solution added, shaken for 10 minutes to extract the oxidised products to the toluene and counted (Hewlett Packard liquid scintillation spectrometer).

Typically, blanks and standards (routinely assayed in triplicate) yielded the following counts per minute:

	BLANK	60 fmol	600 fmol
[NA]	30 - 50	100 - 150	1000 - 1200
[A]	30 - 50	150 - 200	1200 - 1500

Oxidised plasma blanks were not routinely processed as preliminary methodological studies showed similar counts to acid-blanks.

Each completed experiment was always analysed as part of the same assay to avoid interassay variability and allow accurate comparison of catecholamine concentrations before and after an intervention. The intraassay coefficients of variation for replicates were 7% for [NA] and 9% for [A]. Linearity of the assay

for both [NA] and [A] was confirmed over the concentration range measured in venous and arterial plasma (up to 30 pm/ml, Figure 2.1).

From a plasma pool stored at -20°C and assayed at intervals over 400 days, a small but significant trend for decline in measured catecholamines was evident, amounting to 2.7% per month for [NA] and 4.5% per month for [A]. The interassay coefficient of variation over this time was 5% for [NA] and 20% for [A]. Experimental samples were analysed within three months of collection.

LACTATE ASSAY

Lactate concentrations in arterial and venous plasma were determined fluorimetrically according to the method of Passonneau (1970). Briefly, 50 µl plasma (diluted 1:50 for non-ischaemic effluent and 1:100 for ischaemic effluent) is oxidised by lactate dehydrogenase (45 minutes at 37°C) using nicotinamide adenine dinucleotide in the presence of HCl-amino-methyl-propanol buffer and hydrazine hydrate. The resulting reduced nucleotide fluorescence is determined using an Aminco fluorimeter with appropriate blank corrections and read against the standard curve (2, 4, 6, 8, 10 mmol/1).

REGIONAL MYOCARDIAL BLOOD FLOW DETERMINATION

Regional myocardial blood flow was determined by the injection of radioactively labelled microspheres through a left atrial cannula with subsequent analysis of radioactivity in myocardial slices modified from the method of Utley et al (1974).

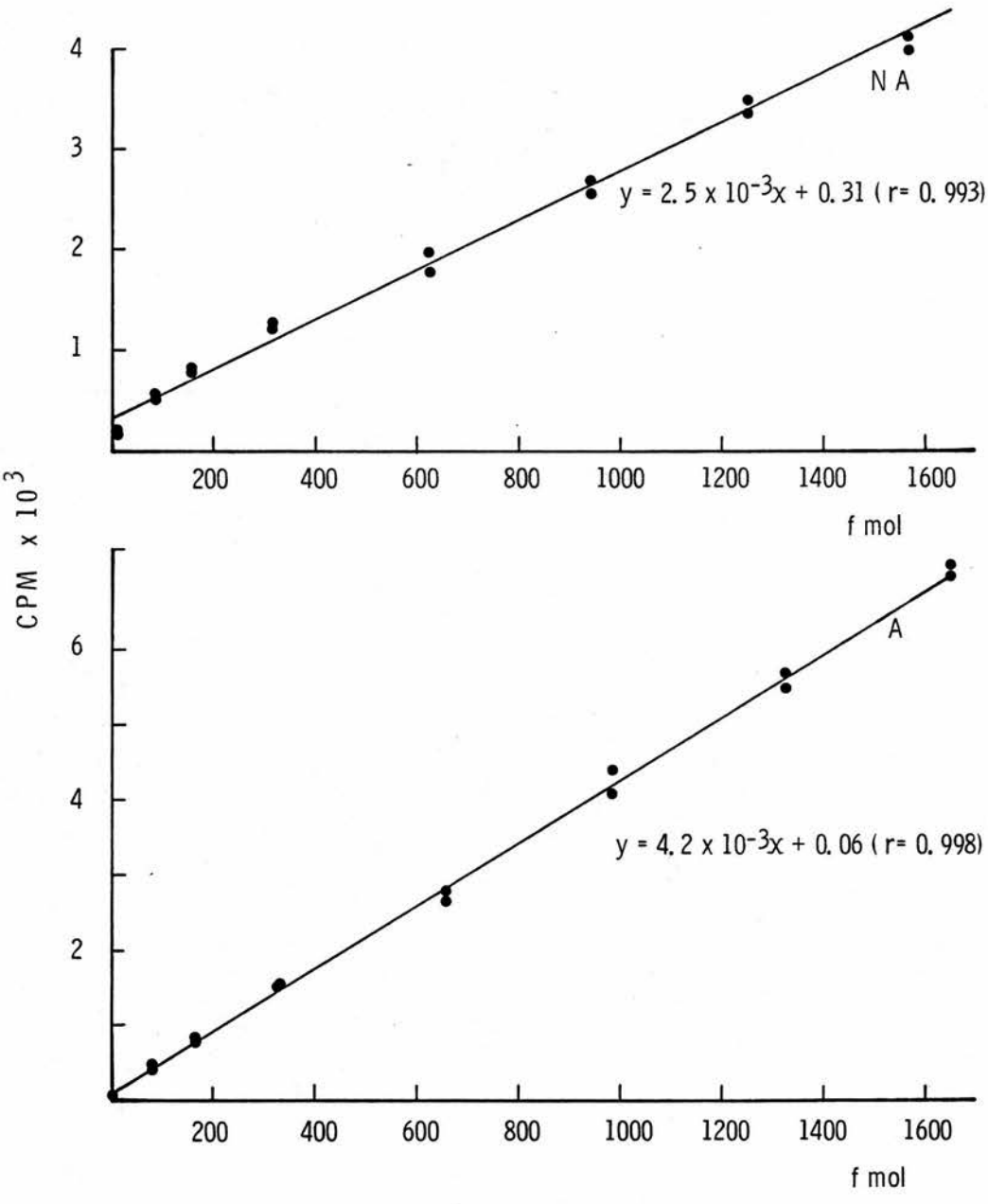


Figure 2.1 Linearity of radioenzymatic assay for l-noradrenaline (NA) and l-adrenaline (A) as free base.

Prior to injection 1.5 x 10^6 (or for detailed mapping studies 8 x 10^6) microspheres, 15 μm in diameter, labelled with Co^{57}, Sn^{133} or Sr^{85}, were suspended in 10 per cent dextran and sonicated for 10 minutes to disperse aggregates before being drawn into a syringe containing 0.1 ml 5% Tween 80 (to prevent reaggregation). The suspension was diluted with 10 per cent dextran to give a final concentration of Tween 80 of less than 0.5 per cent. Microspheres were continually agitated by hand before injection. Ten seconds before injection, withdrawal of a reference blood flow sample from the descending aorta was commenced at constant rate (9 ml per minute) into a pre-weighed heparinised syringe and continued for exactly two minutes. Microspheres were injected into the left atrium over 10 seconds at predetermined times with respect to coronary occlusion, detailed in individual experimental protocols.

After completion of each experiment, the heart was excised and stored overnight at 4°C to facilitate its dissection. An anatomical diagram of coronary anatomy, site of ligation and area of cyanosis was drawn immediately before excision. The free wall of the left ventricle was removed, dissected free of epicardial fat, major blood vessels and papillary muscle and divided into approximately twenty biopsies of relatively constant geometry, including the entire ischaemic area. Samples were further divided into endocardial and epicardial halves and weighed separately. For more detailed blood flow mapping studies using the higher dose of microspheres, the free left ventricular wall was divided into approximately eighty biopsies which were further separated into endocardial and epicardial halves before being weighed (100 - 250 mg).

Radioactivity in each biopsy, and total radioactivity in the reference blood flow sample were determined initially using a two-channel gamma counter (Wallac, LKB-Wallac, Finland) and subsequently using a multichannel gamma counter (Compugamma, LKB-Wallac, Finland) with appropriate windows and energy peak crossover and decay corrections.

Regional myocardial blood flow was calculated for each biopsy from the formula:

$$RMBF = \frac{CM}{CR} \times RBF \quad (ml/min/g)$$

where CM = radioactivity (cpm/g wet weight) in tissue sample

CR = total cpm in reference blood sample

RBF = reference blood flow (ml/min) derived from withdrawal time and volume of blood in reference syringe.

Reproducibility studies have confirmed no systematic differences in measured regional flow with the isotopes used and no significant errors attributable to geometric factors or decay during counting (Riemersma, 1979). Results were calculated using a computer programme and presented as 1) endocardial blood flow, 2) epicardial blood flow and 3) the endocardial-epicardial blood flow ratio.

During experiments infusing ischaemic metabolites without coronary occlusion, continuous measurement of regional (left anterior descending and circumflex) blood flow was carried out using an electromagnetic flowmeter (Skalar Instruments), with 1.5, 2 or 2.5 mm probes, according to vessel diameter. Great care was

taken during the experiment to maintain the probe at right angles to the artery and to minimise movement artefact between vessel and probe. Reproducible changes in blood flow could be achieved following left stellate ganglion stimulation at all frequencies. This technique was used only during the regional infusion of metabolites without coronary occlusion.

REGIONAL EPICARDIAL ACTIVATION TIMES

A 5 x 4 cm flexible multielectrode grid was sutured to the epicardium over the free left ventricular wall across the ischaemic area created by coronary occlusion. This grid provided mapping of the epicardial activation sequence within the ischaemic zone by electronic multiplexing of unipolar electrograms from the 80 point grid. Each electrode was approximately one mm in diameter, separated by 5 mm and grouped in an 8 x 10 array. Signals from each electrode site were passed to a lead selection unit allowing selection of any individual electrogram with respect to the Wilson central terminal. A switch on the lead selection unit allowed the sequential selection of six groups of 14 electrograms (approximately four complexes per group) which were recorded on magnetic tape (4-channel recorder) after passage through a 3-channel multiplexing device coding 5 signals per channel with a sampling frequency of less than 3 ms. Regional epicardial activation time was calculated at each electrode site by replay of the multiplexed signals through a purpose built demultiplexer (Department of Medical Physics, University of Edinburgh) and amplifier and display of individual electrograms on a 3-channel recorder (Mingograf, Siemens Elema). Local conduction delay was derived from the midpoint of the intrinsic deflection of individual

electrograms, compared to the earliest ventricular activation from an electrode in the non-ischaemic area (after Russell et al, 1982).

Conduction delay at each electrode could be reliably measured to the nearest 4 ms (paper speed 250 or 500 mm/sec) and conduction delay maps were constructed at intervals during ischaemia, immediately after low and high frequency left stellate ganglion stimulation and during coronary reperfusion. Electrograms without a clear cut intrinsic deflection, or those with poor quality signals were not included in the analysis. Occasionally, with activation delays greater than 60 ms, the QRS complex became slurred with multiple negative deflections. Under these circumstances, measurements were taken to the midpoint of the major negative deflection of the electrogram, the time most likely to coincide with the upstroke of the local cardiac action potential. The recording and analytical apparatus are shown diagramatically in Figure 2.2.

REGIONAL SAMPLING FROM ISCHAEMIC EFFLUENT

As discussed earlier, regional sampling of ischaemic coronary venous effluent from an ischaemic area depends on equilibration between coronary venous blood and that perfusing the ischaemic territory. This, in turn, is dependent upon the extent to which arterial collaterals from the LAD (proximal to the site of occlusion) and from the circumflex coronary artery perfuse ischaemic or non-ischaemic tissue before draining to the venous sampling site. Clearly the lower the flow in the central ischaemic area, the greater is the potential contribution of non-equilibrated blood to the local coronary venous effluent, either as drainage

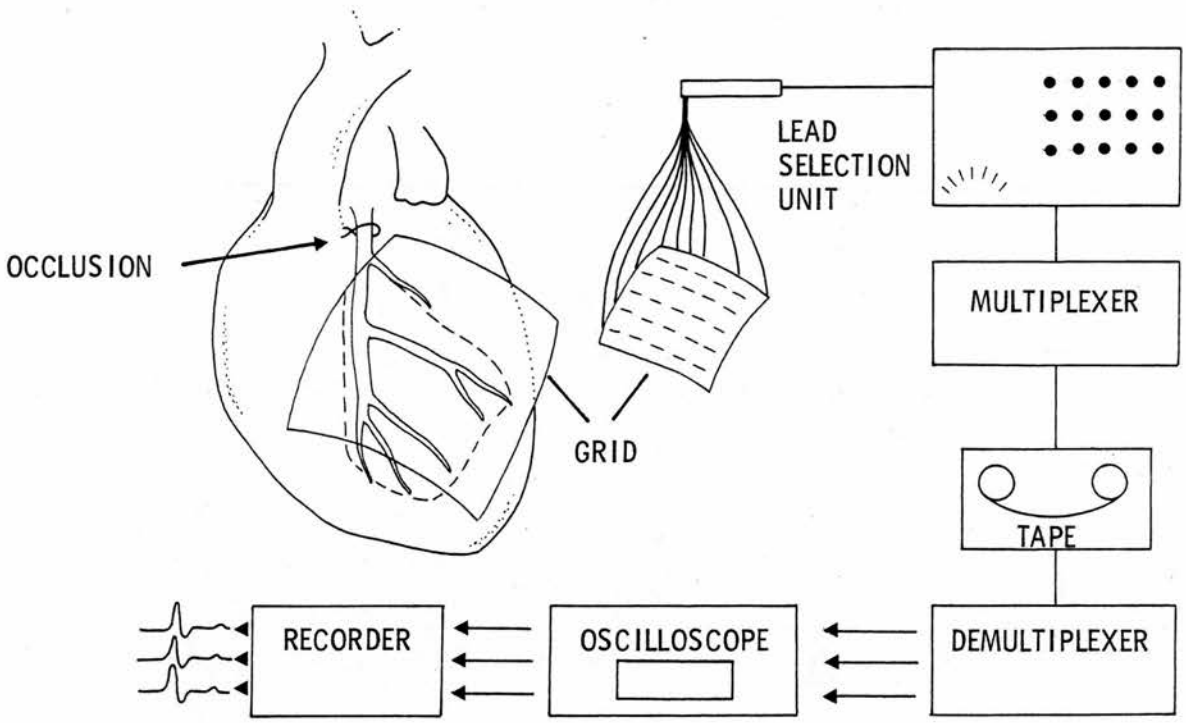


Figure 2.2 Measurement of regional activation time. A flexible multi-electrode grid over the free left ventricular wall and ischaemic area allows sequential recording of individual electrograms at all sites over a few seconds using a multiplexing device (see text).

from a non-ischaemic area or from arteriovenous shunts.

In practice, the contribution of the LAD to perfusion of the ischaemic region can be considered to be nil, since the LAD has generally been occluded proximal to the origin of the first septal perforator branch. At steady state, therefore, the input and output from the territory of drainage of the coronary vein is the same and may be defined as:

$$\text{flow LV} \times [V] = \text{flow LAD} \times [A] + \text{flow } x_1 \times [A] + \text{flow } x_2 \times [A] \quad (1)$$

where flow LV and flow LAD represent local venous and left anterior descending coronary arterial flow respectively, [V] and [A] represent local venous and coronary arterial concentrations respectively of a tracer not extracted or metabolised by the heart; flow x_1 represents the contribution of collateral nutritional flow from the circumflex artery to local venous flow i.e. that component that has equilibrated with the region of interest; and flow x_2 represents the contribution of non-nutritional flow from a non-ischaemic area to local venous flow i.e. that component that has <u>not</u> equilibrated with the region of interest.

During ischaemia as a result of LAD occlusion, flow LAD = 0. Thus, substituting in equation (1), and rearranging

$$\text{flow LV}' = \frac{(\text{flow } x'_1 + \text{flow } x'_2)\,[A]}{[V]} \quad (2)$$

where flow LV', flow x'_1 and flow x'_2 represent the flows defined above during LAD ligation. [A] should be similar to concentrations in the coronary sinus [CS] if the tracer is at steady state and if extraction or metabolism of the tracer is not modified by coronary occlusion. If the circumflex coronary artery is the major

contributor to local venous flow during ischaemia, then [A], [CS] and [LV] should become similar at steady state.

The contributions of nutritional and non-nutritional flow to total venous flow may be derived from the following three principles:

(1) Nutritional flow in the ischaemic area can be measured conventionally using radiolabelled microspheres.

(2) Total venous flow (nutritional and non-nutritional) may be measured directly in an epicardial coronary vein.

(3) Retrograde coronary venous infusion of microspheres can define the territory of drainage of the local vein from which total nutritional flow can be derived (from (1), above).

Source of collateral perfusion in ischaemic area and origins of ischaemic effluent

Preliminary experiments evaluated three extravascular space markers; technetium (Tc^{99m}), I^{131}-hippurate and Xenon. The collection and handling of Xenon in plasma samples proved difficult. I^{131}-hippurate was extracted to a minor degree across the heart (< 10 per cent) but steady state was achieved within 5 minutes. Tc^{99m} extraction averaged 1 per cent across the anterior myocardium and 3 per cent across the circumflex region (coefficient of variation 6 per cent for each), neither significantly different from zero.

In further experiments the effect of retrograde venous

perfusion (in vitro) on labelling of the venous territory with microspheres was evaluated using 25 cm and 75 cm water perfusion pressure. The epicardial area labelled was similar at both perfusion pressures in two studies using paired microsphere injections, although endocardial labelling was 20 per cent greater at the higher pressure. In view of this, the higher pressure was used. At 75 cm water, no significant loss of arterially injected microspheres (wedged in the capillary bed) was observed. Myocardial tissue containing 2 per cent of the total venous injection or greater was arbitrarily designated as part of the territory draining into the local vein.

The studies with ischaemia were performed in open-chest pentobarbitone anaesthetised dogs prepared as described in Chapter 3. The LAD was dissected for occlusion and a venous sampling catheter was placed in the centre of the LAD territory. For this study, a large bore (5FG) portex cannula was used and tied directly into the local coronary vein [LV] occluding its lumen. A lateral catheter towards the coronary sinus [CS] was also inserted conventionally. Circumflex coronary artery flow was measured using an electromagnetic flow probe, and this vessel perfused from the left carotid artery at measured flow as illustrated in figure 2.3. Flow was kept constant through the experiment. Tc^{99m} or I^{131}-hippurate was infused at constant rate proximal to the coronary perfusion pump and blood sampled just prior to the circumflex artery [A]. Tracer concentrations (cpm/ml) at [A], [LV] and [CS] sampling sites were measured at minute intervals over 20 minutes, ten minutes before and ten minutes during LAD occlusion. Flow in the local venous territory was measured directly by timing blood draining from the [LV] catheter, used as a syphon.

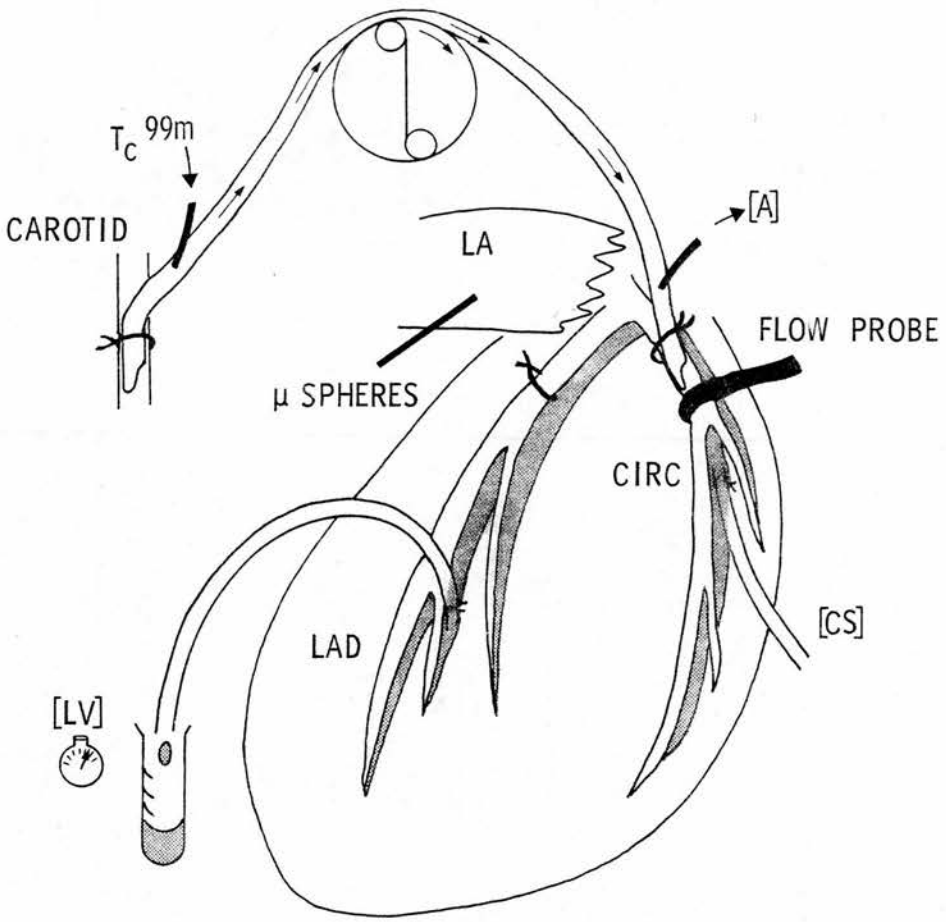


Figure 2.3 Validation of local venous sampling: experimental preparation. The circumflex coronary artery was perfused at constant flow from the carotid artery and labelled selectively using tecnetium (Tc) 99m. Nutritional flow was measured from intraatrial microsphere injection and local venous (LV) flow measured directly (see tcxt).

Nutritional blood flow was determined 5 minutes after LAD occlusion using intraatrial microspheres as described earlier.

At the end of each experiment, the heart was carefully excised and supported in a cradle. Microspheres (20-50,000) were infused into the local venous catheter and flushed over 10 minutes at constant pressure (75 cm H_2O). The free left ventricular wall was then prepared as described previously and cut into 36 biopsies including the whole of the LAD territory. Each biopsy was further divided into endocardial and epicardial regions by midline dissection and counted. Biopsies containing greater than 2 per cent of the venous tracer (from in-vitro labelling) were designated as part of the region drained by the local vein and mean nutritional flow and then determined as described previously.

As shown in Figure 2.4 in a representative experiment, tracer levels in [LV] increased rapidly following LAD occlusion and were not significantly different from [CS] concentrations during acute ischaemia. In this study, therefore, the circumflex coronary was the source of blood flow to the ischaemic region. If the LAD or right coronary had contributed significantly, the concentrations in the [LV] would have remained below those in the [CS]. In two other experiments, [LV] concentrations averaged 80 per cent of [CS] concentrations during ischaemia.

Using this model, mean nutritional flow in the region of interest (as defined above) in three experiments was 1.3, 1.0 and 0.9 ml/min for territories of 4.3, 2.3 and 3.8 g wet weight respectively. Venous flow, averaged over 2-8 minutes of ischaemia, which included the period of microsphere injection, was 2.0, 1.2 and 1.5 ml/min. Nutritional flow averaged 65, 83 and 60 per cent

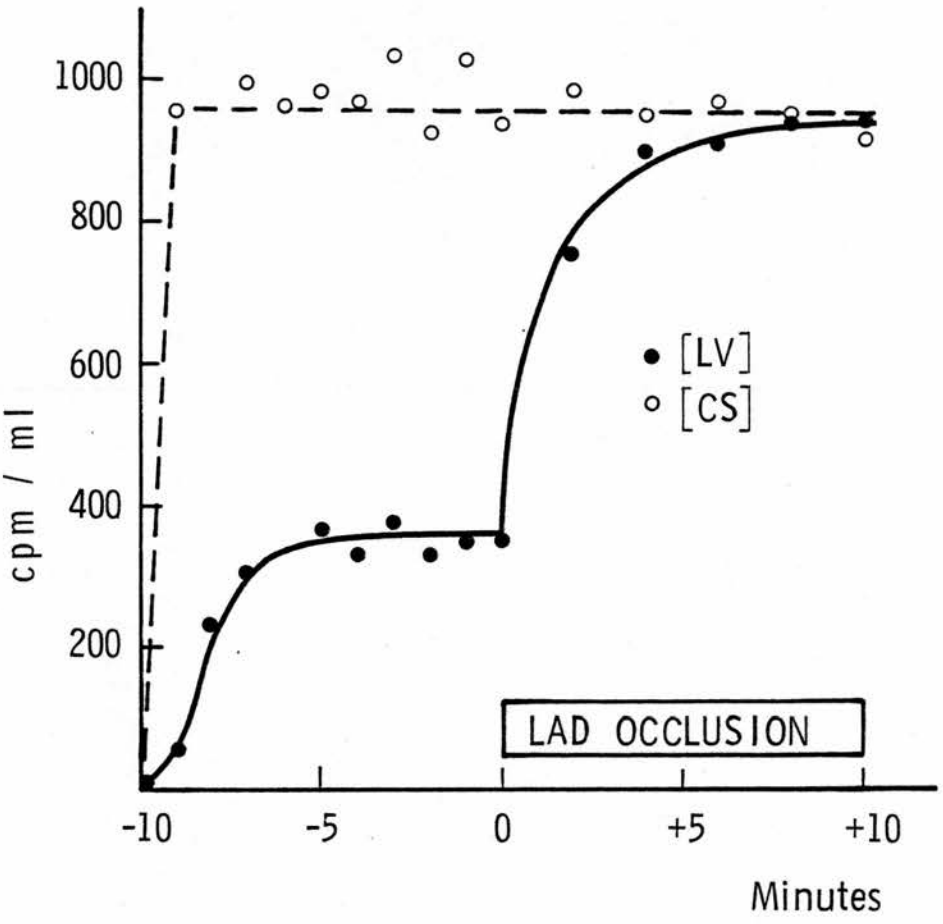


Figure 2.4 Effects of LAD occlusion on anterior (LV) and lateral (CS) venous concentrations of I^{131}-hippurate during infusion into the circumflex coronary artery. See text for details. Data from one experiment.

of total venous flow in [LV].

Thus, in this model, despite several assumptions, the bulk of coronary venous effluent has indeed perfused the ischaemic region created by LAD occlusion. The use of regional venous sampling can therefore be considered to reflect qualitatively changes in metabolites in the ischaemic area. It is likely, however, that hormone and metabolite spillover into coronary venous effluent will occur to a greater extent from moderately ischaemic rather than severely ischaemic regions, although this has not been quantified. On the other hand, however, a small contribution to local venous effluent from non-ischaemic or non-equilibrated areas precludes the use of this technique to quantify output from the ischaemic region. For this reason, results in this thesis have generally been expressed in terms of arteriovenous concentration differences rather than output, although regional flow has been measured in all experiments.

3 SPONTANEOUS CATECHOLAMINE RELEASE FROM ACUTELY ISCHAEMIC MYOCARDIUM

It is generally accepted that increased liberation of catecholamines in the heart follows the onset of myocardial ischaemia or infarction. Indeed, few authors appear to consider that the question requires detailed discussion or debate (Hoffman, 1978). However, although the evidence favouring activation of efferent neurosympathetic activity to the heart following acute ischaemia is considerable, much of it is indirect and dependent on observations following pharmacological or surgical manipulation of the cardiac neurosympathetic axis, or on observation of end organ responses rather than the activity of the nerve terminal itself.

In keeping with the clinical evidence summarised in Chapter 1, arterial catecholamine levels rise soon after the onset of experimental coronary occlusion. Staszewska-Barczak and Ceremuzynski (1968) observed a progressive rise in arterial adrenaline over the first 15 minutes of coronary ligation in a majority of anaesthetised dogs, with secretion rates up to 8 µg/min. Arterial noradrenaline levels rose infrequently. Once elevated, arterial catecholamine levels remained constant over a 3-4 hour period, contrasting with the periodicity of spontaneous arrhythmias over this period in this model. Nevertheless, these authors observed a relationship between adrenaline release and spontaneous arrhythmias (Ceremuzynski et al, 1969), and established that adrenaline release occurred from the adrenal medulla as a result of reflex stimulation from cardiac receptors in the centre of and at the periphery of the evolving infarct. This reflex involved vagal efferent as well as extra-vagal pathways and required intact supraspinal structures (Staszewska-Barczak, 1971). The source of the infrequently observed increase in noradrenaline was not identified.

The noradrenaline content of infarcted heart muscle has decreased to less than 40 per cent of control by 24 hours and rapidly declines towards zero thereafter (Gudbjarnason, 1972). The rate of decline in tissue catecholamine content during the reversible phase of acute ischaemia has not been assessed. Muntz et al (1981) noted no significant reduction in tissue catecholamine content one and three hours after LAD ligation in dogs, but after three hours these authors noted a significant reduction in catecholamine fluorescence within sympathetic nerves, suggesting that catecholamines were redistributed within ischaemic myocardium at this time. Russell et al (1961) noted a small reduction in infarcted tissue noradrenaline content (from 0.83 $\pm$ 0.32 to 0.64 $\pm$ 0.16 µg/g wet weight) one hour after coronary artery ligation in the dog. No significant reduction in noradrenaline content in rat ventricular myocardium was noted 30 minutes after left coronary artery ligation (Abrahamsson et al, 1982). In non-infarcted myocardium, a progressive decline in noradrenaline content was observed over the first two weeks following coronary ligation, with a gradual recovery of normal tissue levels over a further four weeks (Gudbjarnason et al, 1971). Neither time course bears a close relationship to spontaneous arrhythmias in this model. As measurement of tissue catecholamines does not distinguish between intraneuronal (stored) and extraneuronal (released) neurotransmitter, such studies are unlikely to define nerve terminal activity.

A reduced threshold for ventricular arrhythmias after catecholamine infusion during experimental infarction has been known for over 20 years (Maling and Moran, 1957; Winbury et al, 1962; Cha et al, 1970), with a time course rather similar to

depletion of noradrenaline in the infarct zone. These studies avoided to a large extent the early phase of arrhythmias (Harris, Phase I) by using a two-stage coronary ligation procedure (Harris, 1950) and therefore cannot provide information on sensitivity to catecholamines during acute ischaemia (ligation for 10-20 minutes) as opposed to infarction (ligation beyond 24 hours).

Catecholamine perfusion of isolated hearts produces several metabolic and ultrastructural abnormalities similar to acute ischaemia. Thus, depletion of tissue ATP and glycogen, accumulation of lactate, leakage of creatinine phosphokinase and aspartate aminotransferase have been demonstrated with myofibrillar abnormalities and mitochondrial rounding, enlargement and altered electron density (Williamson, 1964, 1966; Waldenstrom et al, 1978). In general, the concentrations of catecholamines used for such studies have been approximately two to three orders of magnitude higher than those seen under physiological conditions. However, local myocardial concentrations round the nerve terminal may be many times greater than in plasma, particularly if neurosympathetic activity has been stimulated by ischaemia, and removal mechanisms impaired. Similar harmful metabolic effects of endogenous catecholamines on the development of hypoxic myocardial cell damage in the rat have been shown by Gauduel et al (1979).

The purpose of the present study was to observe on a minute to minute basis catecholamine concentrations in arterial and in coronary venous blood draining ischaemic and non-ischaemic myocardium during experimental acute coronary ligation before, at the onset of, and following spontaneous ventricular arrhythmias including ventricular fibrillation. Tritiated noradrenaline is

rapidly taken up by the dog heart and equilibrates with the intraneuronal stored neurotransmitter pool (Chidsey et al, 1963; Fillon et al, 1971). The first demonstration of labelled noradrenaline release with nerve stimulation was made by Hertting and Axelrod in 1961. Since then, exogenous adrenergic transmitter release from the heart has been correlated with a variety of physiological and pathophysiological responses in the dog model in vivo (Gaffney et al, 1963; Harrison et al, 1963; Yamaguchi et al, 1976). Release of prelabelled noradrenaline from the myocardium into the coronary sinus increases following intracoronary tryamine and left stellate ganglion stimulation (Yamaguchi et al, 1973). Catecholamine release from the heart occurs at physiological nerve stimulation frequencies (1-3 Hz) despite intact uptake mechanisms, suggesting that the venous catecholamine content is a reflection of changing levels at the nerve terminal (Yamaguchi et al, 1975). This increased production is independent of haemodynamic changes (Siegel et al, 1961). The studies reported in this thesis are therefore based on the assumption that changes in nerve terminal catecholamine release from alterations in efferent sympathetic activity or from local myocardial influences on the nerve terminal itself are reflected by corresponding changes in venous catecholamine content.

[Bronk et al, 1936]

METHODS

Operative and Sampling Methods

After an overnight fast, adult mongrel dogs (10-24 kg) of either sex were anaesthetised with 50 mg/kg intravenous alpha-chloralose in urethane (25%) thirty minutes after

premedication with 2 mg/kg intramuscular morphine (Series I). Maintenance anaesthesia was achieved by constant intravenous infusion of 5 mg/kg/hour alpha-chloralose in urethane. For Series II, morphine premedication was omitted and anaesthesia induced by 100 mg/kg alpha-chloralose alone as a slow intravenous infusion. Maintenance was achieved by 10 mg/kg/hour alpha-chloralose. Following endotracheal intubation, animals were ventilated on room air using a positive pressure Harvard respirator at 15 cycles per minute and appropriate tidal volume.

The operative procedures were as follows: sampling, infusion and monitoring catheters (Portex 3FG, 6FG and 8FG respectively) were inserted into both right and left femoral vessels, and the heart was exposed through a left lateral thoracotomy (fifth interspace) and suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated within 3 cm of its origin and a loose ligature placed round it to facilate occlusion using a Mayfield intracranial arterial clip. Great care was taken during the dissection to minimise injury to pericoronary nerves which were readily visualised. Two further catheters (3FG) were inserted into epicardial coronary veins, one antegradely towards the centre of the LAD territory and the other retrogradely into a vein draining circumflex territory effluent towards the coronary sinus. These catheters were similar to the arterial sampling catheter except for two side holes, cut into the side of the catheter within 2 cm of its tip to facilate sampling at low flow rates. During insertion, the catheters were continuously flushed with saline to prevent clotting and when in position were sutured to the epicardium and filled with heparin(1000 iu/ml). A left atrial catheter (3FG) was inserted via the atrial appendage

for microsphere injections.

For intracoronary arterial infusions (morphine-chloralose-urethane anaesthesia), a small (approx 1-1.5mm external-diameter) side branch of the proximal LAD was isolated as it crossed the root of the pulmonary artery towards the right ventricle. Occasionally, this vessel proved too small and therefore the first small proximal LAD branch to the right or left of the septum was used. A Portex catheter (1FG) was heparinised and inserted such that its tip lay just at the point of bifurcation of the vessel from the LAD but without projecting into the lumen of the parent vessel. For technical reasons, this procedure was unsuccessful in three animals, who were subsequently assigned to the main experimental protocol. The experimental preparation is shown in Figure 3.1.

In four experiments (morphine-chloralose-urethane anaesthesia), the left fifth rib was resected, the lung retracted and the anterior and posterior ansa subclavia from the left stellate ganglian dissected free from fat and subcutaneous tissue and attached to a silver bipolar stimulating electrode for stimulation using a Grass model S48 stimulator. No coronary dissection was performed for these studies but coronary venous sampling catheters were inserted as described above.

In each experiment following completion of surgery, the preparation was heparinised (100 u/kg) and allowed to recover for 30 minutes or until haemodynamically stable. Arterial blood pressure (Elcomatic 751 transducer), heart rate and the surface ECG were recorded continuously on a Devices recorder. Ventricular fibrillation during coronary occlusion was managed by removal of

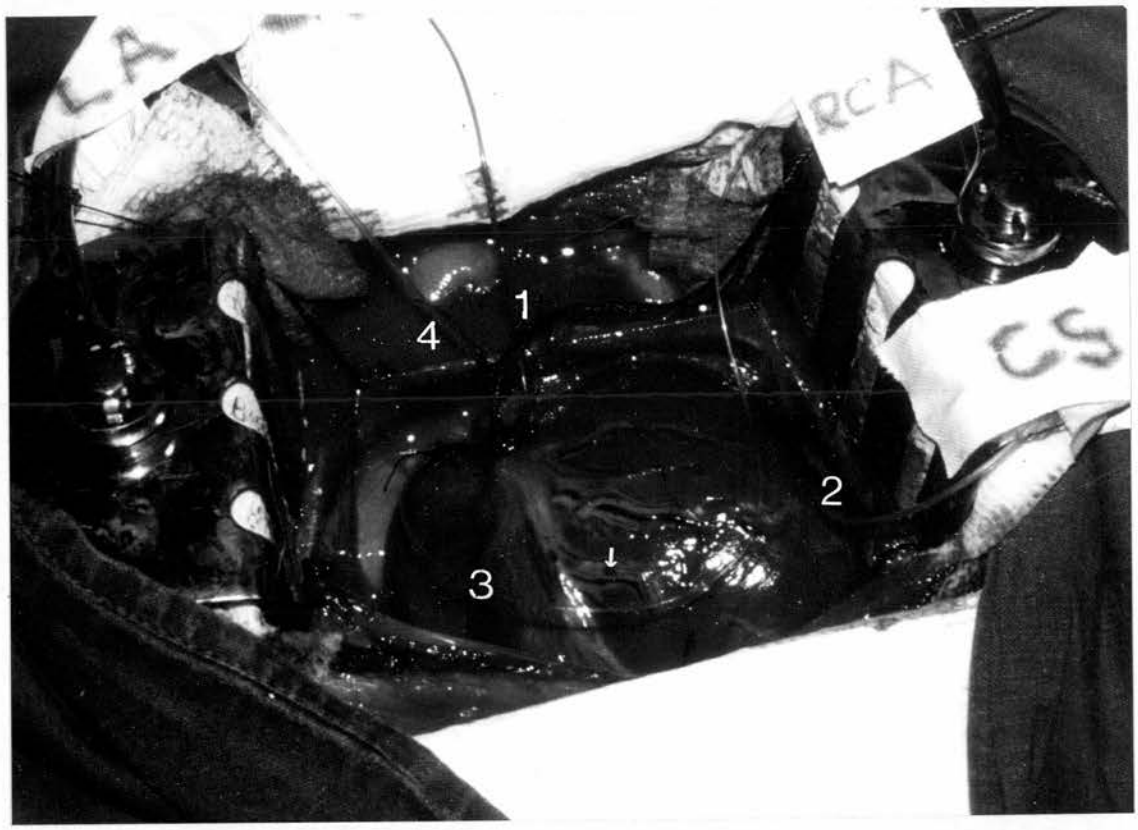


Figure 3.1 Experimental preparation. Sampling catheters in a local vein in the centre of the LAD territory (1, tip arrowed) and laterally draining towards the coronary sinus (2) allow partial separation of ischaemic and non-ischaemic effluent. Infusion catheters in a small side branch of the LAD (3) and in the left atrium (4) are also shown. A loose ligature is round the LAD at the site of occlusion.

the occlusion clip and DC defibrillation (10–20 joules) repeated as necessary.

Series I:

The effect of coronary occlusion on arterial and cardiac venous catecholamines was initially studied in 12 experiments (Series I). Thirty minutes after completion of surgery, and prior to coronary artery occlusion coronary venous effluent was sampled continuously for ten minutes from the local vein in the LAD territory using a calibrated Watson-Marlow roller pump at a flow rate of 1 ml/min). Arterial samples were collected from the aorta at five minute intervals.

Coronary venous and arterial sampling were then continued during a ten minute period of LAD occlusion and for ten minutes of reperfusion following abrupt release of the occlusion clip. Regional myocardial blood flow was estimated by microsphere injection 5 minutes after LAD occlusion (Figure 3.2a). One experiment was subsequently excluded from statistical analysis because of excessive variability in depth of anaesthesia and correspondingly high basal catecholamine levels.

Handling of prelabelled noradrenaline by ischaemic myocardium

The effect of coronary occlusion on the efflux of dl ^{3}H-noradrenaline and its metabolites from the ischaemic area was studied in five experiments. Prior to coronary occlusion, 25 µCi dl ^{3}H-noradrenaline was infused into the LAD over ten minutes, the infusion being completed 15 minutes before the start of sampling.

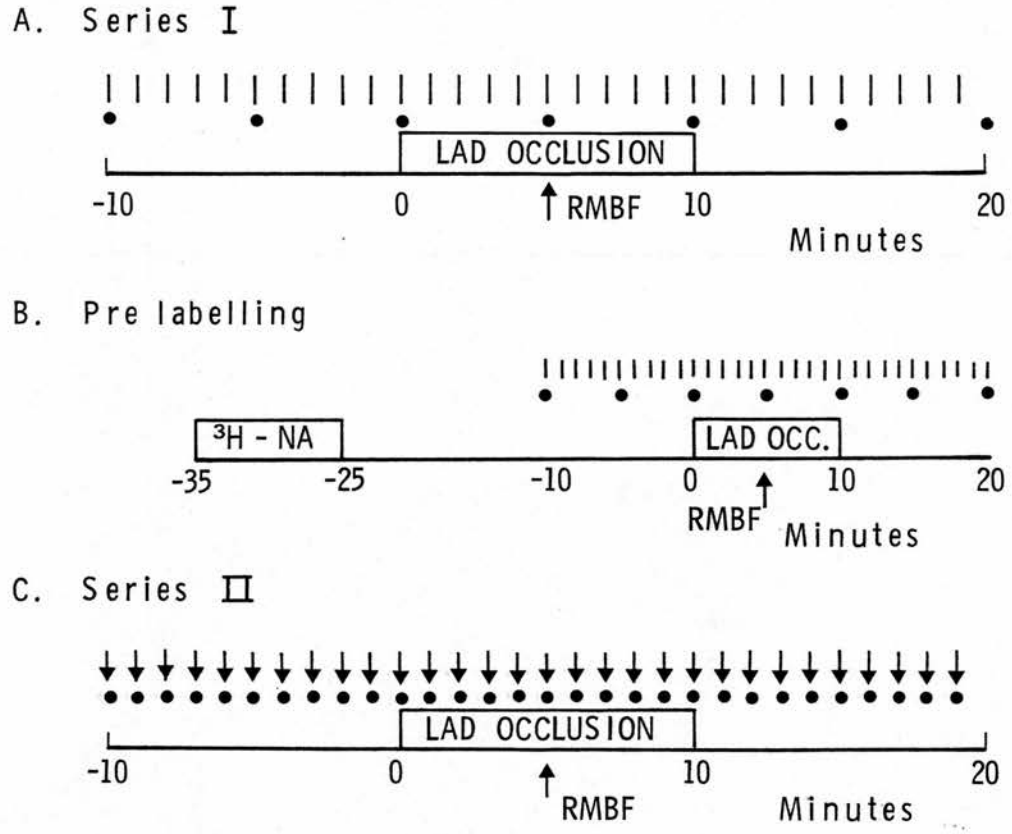


Figure 3.2 Experimental protocols; spontaneous catecholamine overflow during acute ischaemia.

Local venous and arterial samples were collected as in Series I for 10 minute periods before, during and after LAD occlusion (Figure 3.2b) and plasma (100 µl) counted directly in a liquid scintillation spectrometer. In four of these experiments, the catecholamine content of venous and arterial samples was also determined enzymatically, forming part of Series I. The object of these experiments was to determine whether catecholamines released from sympathetic nerve terminals during acute ischaemia were metabolised prior to their efflux into coronary venous effluent.

Series II:

The basic experimental design in this series of 16 experiments using α-chloralose anaesthesia was similar with the addition of continuous sampling from arterial and coronary sinus catheters (Figure 3.2c). This anaesthetic agent was used alone to avoid possible vagotonic effects of morphine.

Validation of detection of catecholamine release from the heart:

In two studies without LAD occlusion l-noradrenaline dissolved in isotonic saline containing 1% ascorbic acid was infused into the LAD territory in physiological doses (5, 10 and 15 ng/kg/min), each over five minutes with continuous sampling of local venous effluent for five minutes before, during and for five minutes after the infusion (6 experiments). Intracoronary tyramine (5 µg/kg/min) was infused for 15 minutes with continuous local venous sampling for ten minutes before, during and for five minutes after the infusion.

The anterior and posterior ansa subclavia from the left stellate ganglion were stimulated for one minute periods at supramaximal voltage (8v) using square wave pulses (4 msec) at 8 Hz. Continuous sampling of local venous, coronary sinus and arterial plasma before, during and after sympathetic stimulation was undertaken in four experiments.

All plasma samples were analysed for adrenaline ([A]) and noradrenaline ([NA]) as described in Chapter 2. Substantially reduced local venous flow during ischaemia usually decreased the sample volume from the ischaemic area to 500 µl or less. Occasionally, adequate volumes were not obtained over one minute and collection was therefore continued for two minutes before proceeding to the next sample. Plasma lactate was analysed in selected pre-occlusion samples and at intervals during occlusion and reperfusion. Samples obtained following resuscitation were not included in the overall statistical analysis.

Trends in arterial catecholamine concentrations with time were evaluated where appropriate with a linear regression co-efficient and least squares method of analysis. Variability in levels before or during coronary occlusion was evaluated by analysis of variance and computed modified t-statistic. Students t-test for paired data was used to calculate probability values on arteriovenous differences and on the combined results from before, during and after coronary occlusion periods where no differences were observed within each of these periods. Changes in peak myocardial catecholamine release during coronary reperfusion were analysed by comparing levels immediately before to those immediately after release of the occlusion clip. Numerical data are expressed as

		BASAL					OCCLUSION					REPERFUSION				
		2'	4'	6'	8'	10'	2'	4'	6'	8'	10'	2'	4'	6'	8'	10'
NA(pmol/ml)																
Art	$\bar{x}$	1.2		1.3		1.5			1.6		1.9			1.9		2.4
	SEM	0.2		0.1		0.2			0.2		0.2			0.2		0.3
LV	$\bar{x}$	1.8	1.6	1.4	1.9	1.9	1.9	2.3	2.0	1.8	2.2	3.6*	2.3	2.6	2.6	2.4
	SEM	0.2	0.1	0.1	0.2	0.2	0.2	0.4	0.3	0.2	0.3	0.8	0.3	0.3	0.3	0.3
A (pmol/ml)																
Art	$\bar{x}$	1.2		1.2		1.3			3.5+		3.8+			3.0		5.2
	SEM	0.4		0.4		0.4			1.6		1.5			2.0		2.5
LV	$\bar{x}$	0.7	0.8	0.8	0.9	0.9	1.0	0.8	0.9	0.9	1.2	2.9*	3.3	2.9	2.3	1.4
	SEM	0.2	0.2	0.2	0.3	0.3	0.3	0.1	0.2	0.3	0.4	1.1	1.4	1.1	0.8	0.4

* $P < 0.02$ wrt pre-release + $P < 0.05$ wrt basal levels

Table 3.1: Spontaneous catecholamine concentrations before, during and after LAD occlusion for ten minutes (n = 11, Series I).

mean ± standard error of the mean. A five per cent level of confidence was considered significant.

RESULTS

SERIES I

Mean data for plasma catecholamines over two one-minute sampling periods out of the ten minute sampling periods before, during and after coronary occlusion are given in Table 3.1 and shown graphically on a minute-to-minute basis in Figures 3.3 and 3.4. The changes in [NA] and [A] (compared to basal levels) during and after LAD occlusion are given in table 3.1a.

Release of small amounts of [NA] from the heart was observed during the preocclusion period but no substantial or significant changes in concentration followed coronary occlusion, although increased variability in levels in ischaemic venous effluent was observed (Figure 3.3). Increased [NA] concentrations in ischaemic effluent were, however, observed during coronary reperfusion, reaching a peak within the first minute of release of the occlusion clip and declining rapidly towards pre-release levels thereafter. Arterial [NA] increased progressively, levels approximately doubling during the sampling period ($p < 0.005$). A significant increase in regional [NA] release from the control period was observed for the first two minutes of reperfusion only (Figure 3.5).

In contrast, venous [A] levels were lower than arterial levels during the control period (Figure 3.4). Ischaemia caused an immediate three fold rise in arterial [A] ($p < 0.05$ compared to basal levels) but no significant changes in venous [A] indicating

	OCCLUSION			REPERFUSION		
	2'	6'	10'	2'	6'	10'
NA (pmol/ml)						
Art $\bar{x}$	-	0.3	0.7	-	0.7	1.1**
SEM		0.2	0.4		0.4	0.4
LV $\bar{x}$	0.2	0.3	0.4	1.9**	0.7	0.5
SEM	0.2	0.4	0.4	0.6	0.4	0.5
A (pmol/ml)						
Art $\bar{x}$	-	2.4*	2.8*	-	1.9	4.2*
SEM		1.3	1.4		1.6	2.7
LV $\bar{x}$	0.3	0.2	0.6	2.1*	2.0	0.7
SEM	0.1	0.2	0.4	0.9	1.7	0.3

* $p < 0.05$ ** $p < 0.02$ wrt basal level

TABLE 3.1a: Changes in catecholamine concentrations during and after LAD occlusion for ten minutes (n = 11; Series 1). Data from Table 3.1.

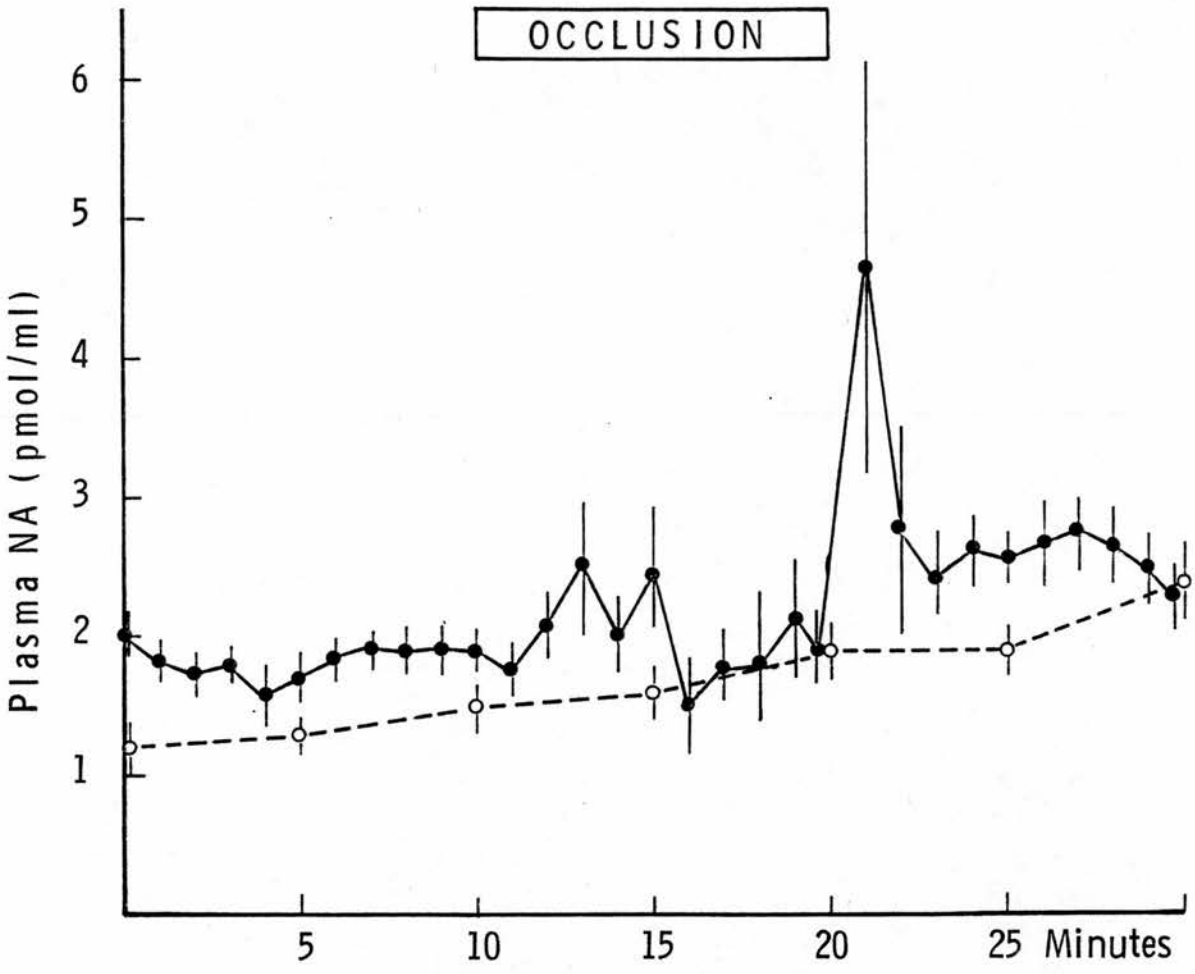


Figure 3.3 Arterial (open circles) and local venous (closed circles) noradrenaline (NA) responses to ten minutes LAD occlusion and reperfusion (Series 1, mean ± SEM).

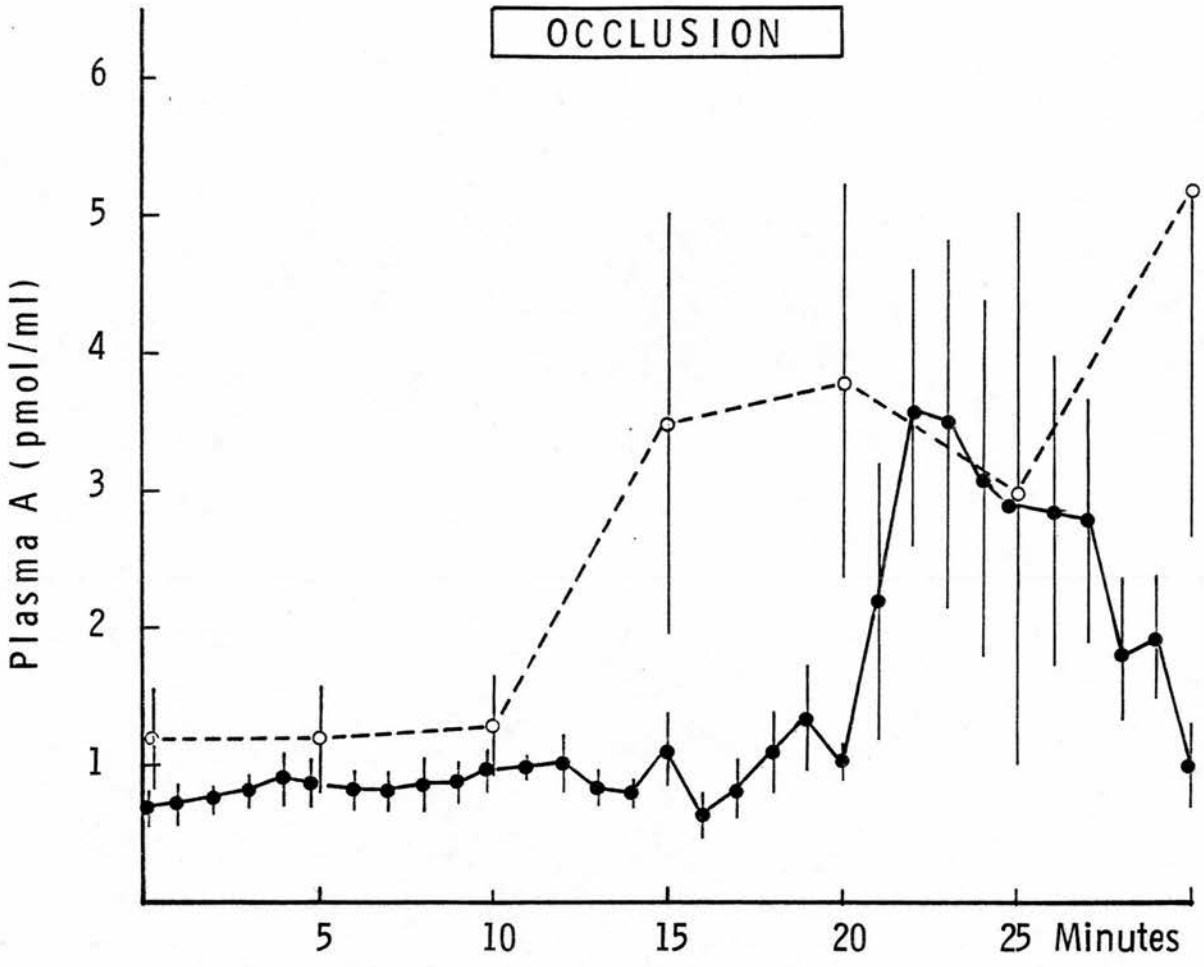


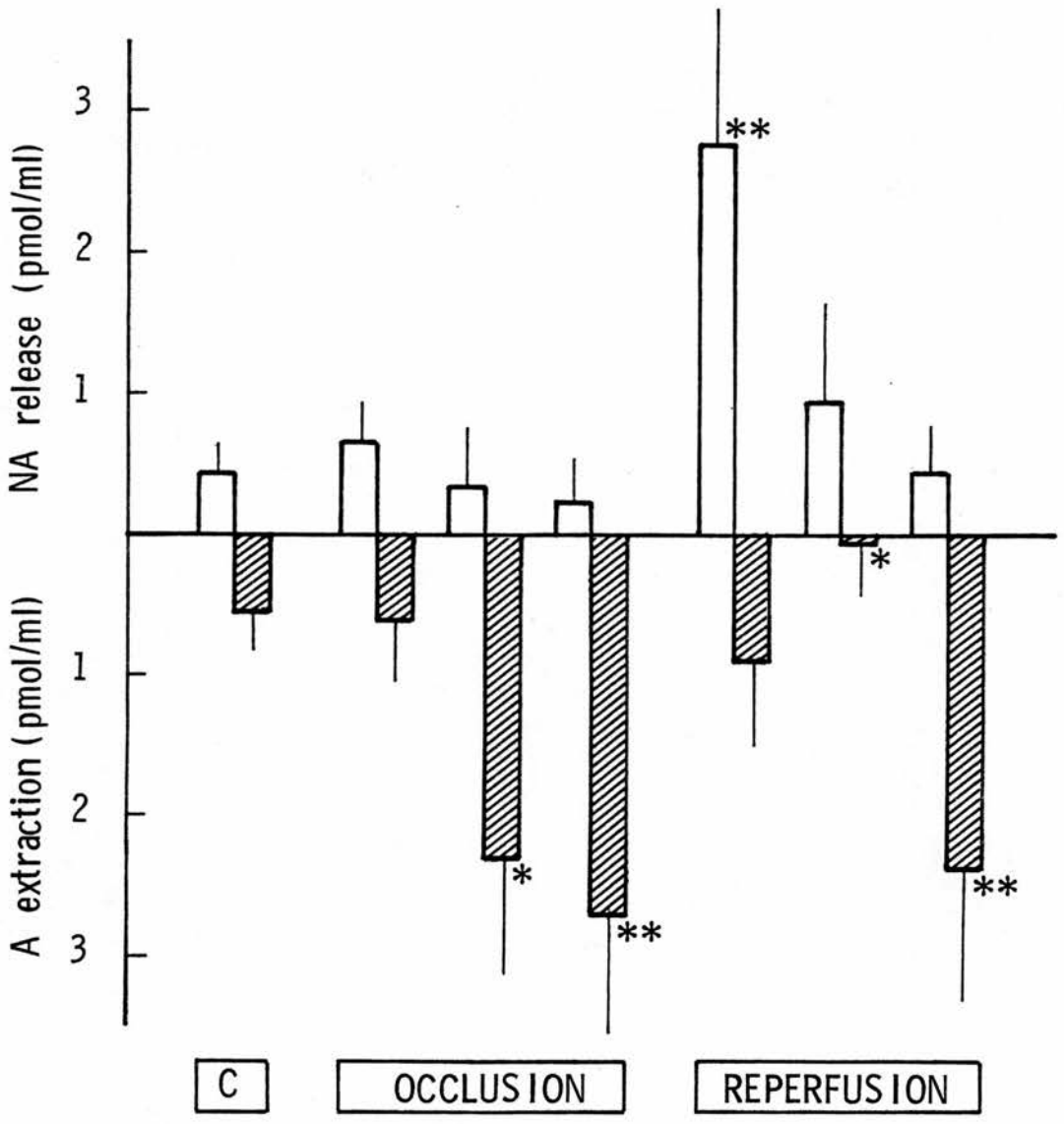
Figure 3.4 Arterial (open circles) and local venous (closed circles) adrenaline (A) responses to ten minutes LAD occlusion and reperfusion (Series I, mean ± SEM).

significantly enhanced extraction of [A] across the ischaemic area (Figure 3.5). Reperfusion increased venous [A] and abolished extraction for 4-5 minutes, a time course rather similar to the period of reactive hyperaemia. It was not possible to know whether actual release of [A] from the heart occurred at this time without more frequent measurement of arterial levels.

Arrhythmias:

Frequent ventricular premature beats (> 10/minute) and/or self terminating ventricular tachycardia occurred in three experiments which form part of the data detailed above. As shown in Figure 3.6, patterns of arterial and venous catecholamines and in particular, levels immediately preceding the arrhythmias, did not differ substantially in these experiments, from the group as a whole. Reperfusion-induced release of [NA] was seen in two of the three studies, in one of which (Figure 3.6b) release was delayed for four minutes after removal of the occlusion clip. In this experiment, a similar delay in the appearance of reactive hyperaemia was noted, suggesting that mechanical factors at the occlusion site or possibly vasospasm may have delayed the washout phase associated with reperfusion. A small but statistically significant increase in local venous [NA] with coronary occlusion was noted in one study (Figure 3.6c) during frequent ventricular premature beats without fibrillation (control [NA] 0.7 $\pm$ 0.05 pmol/ml; occlusion [NA] (during VPB's) 1.6 $\pm$ 0.05 pmol/ml, $p < 0.01$). Arterial [NA] remained low during this study but transient increases in arterial levels during the phase of arrhythmias were not excluded.

Spontaneous ventricular fibrillation occurred in three



* $p < 0.05$ wrt control
** $p < 0.01$ wrt control

Figure 3.5 Noradrenaline (NA) release from and adrenaline (A) extraction across ischaemic myocardium (2, 6, 10 min) and on reperfusion. Control data (C) also shown (from table 3.1).

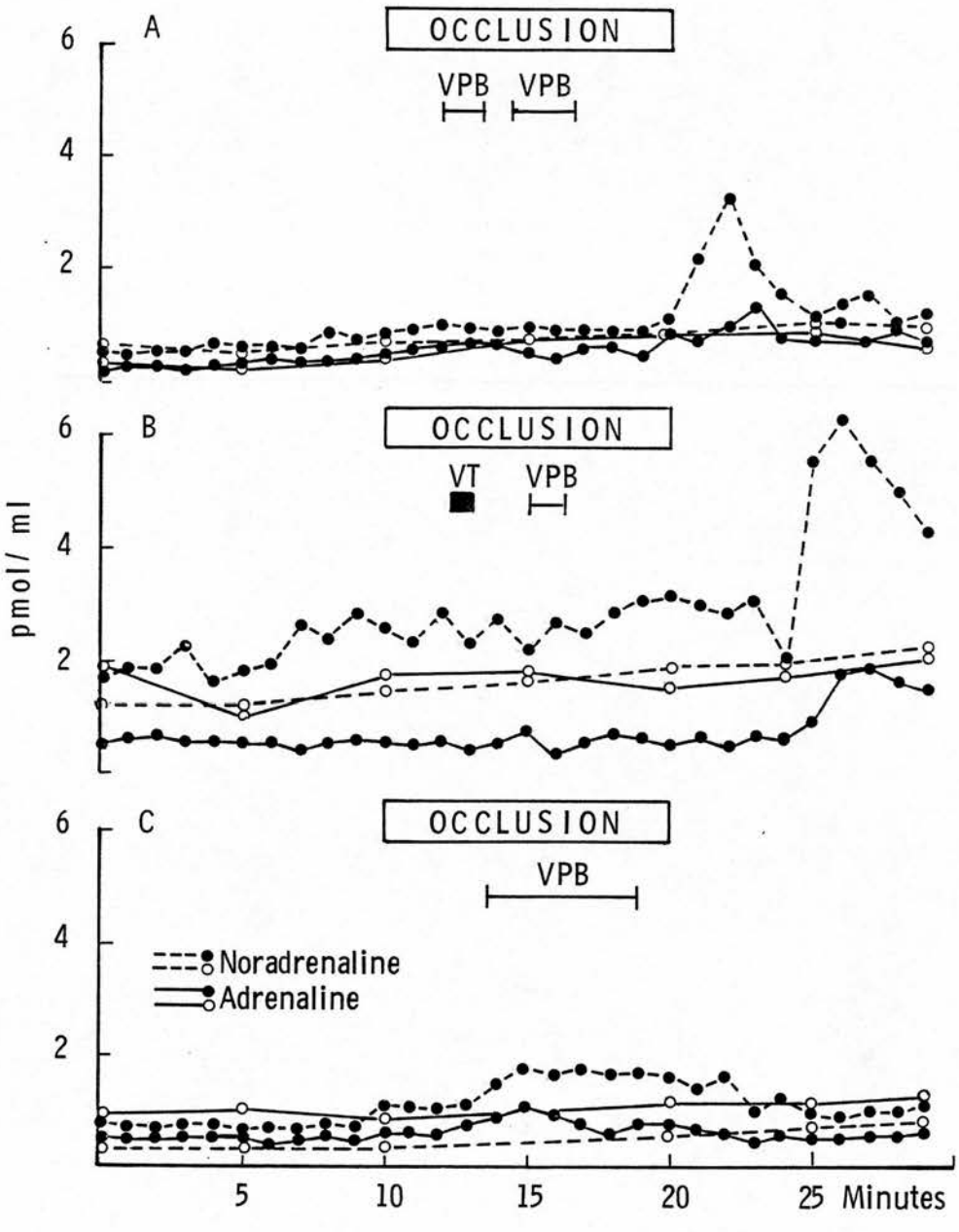


Figure 3.6 Arterial (open circles) and local venous (closed circles) catecholamine responses during ischaemia complicated by frequent ventricular premature beats (VPB) or self terminating ventricular tachycardia (VT). Three experiments.

experiments once during LAD occlusion and twice within the first few seconds of reperfusion. Local venous [NA] increased significantly, immediately before spontaneous ventricular fibrillation (Figure 3.7a). Arterial [NA] was also increased when first measured following defibrillation and both levels returned to preocclusion values over five minutes. Haemodynamically significant ventricular tachycardia occurred prior to spontaneous ventricular fibrillation. Reperfusion induced ventricular fibrillation (Figure 3.7b) did not modify the pattern of [NA] release and abolished [A] extraction associated with this manoevre in the absence of the arrhythmia.

Metabolites:

Prelabelling of the ischaemic territory with ^{3}H-noradrenaline did not reveal consistent efflux of [NA] or its metabolites from the ischaemic area (Figure 3.8a). Trend analysis of the decrease in counts over time did not reveal statistically significant differences between the occlusion and preocclusion periods (pooled results). Two studies, however, did suggest some increase in radioactivity in ischaemic effluent, the first immediately preceding spontaneous ventricular fibrillaton (Figure 3.8b) and the second during the development of ventricular premature hearts (Figure 3.8c). In both of these experiments, arterial counts declined normally, indicating absence of generalised sympathetic activation over this time.

SERIES II

The overall pattern of [NA] and [A] responses to acute

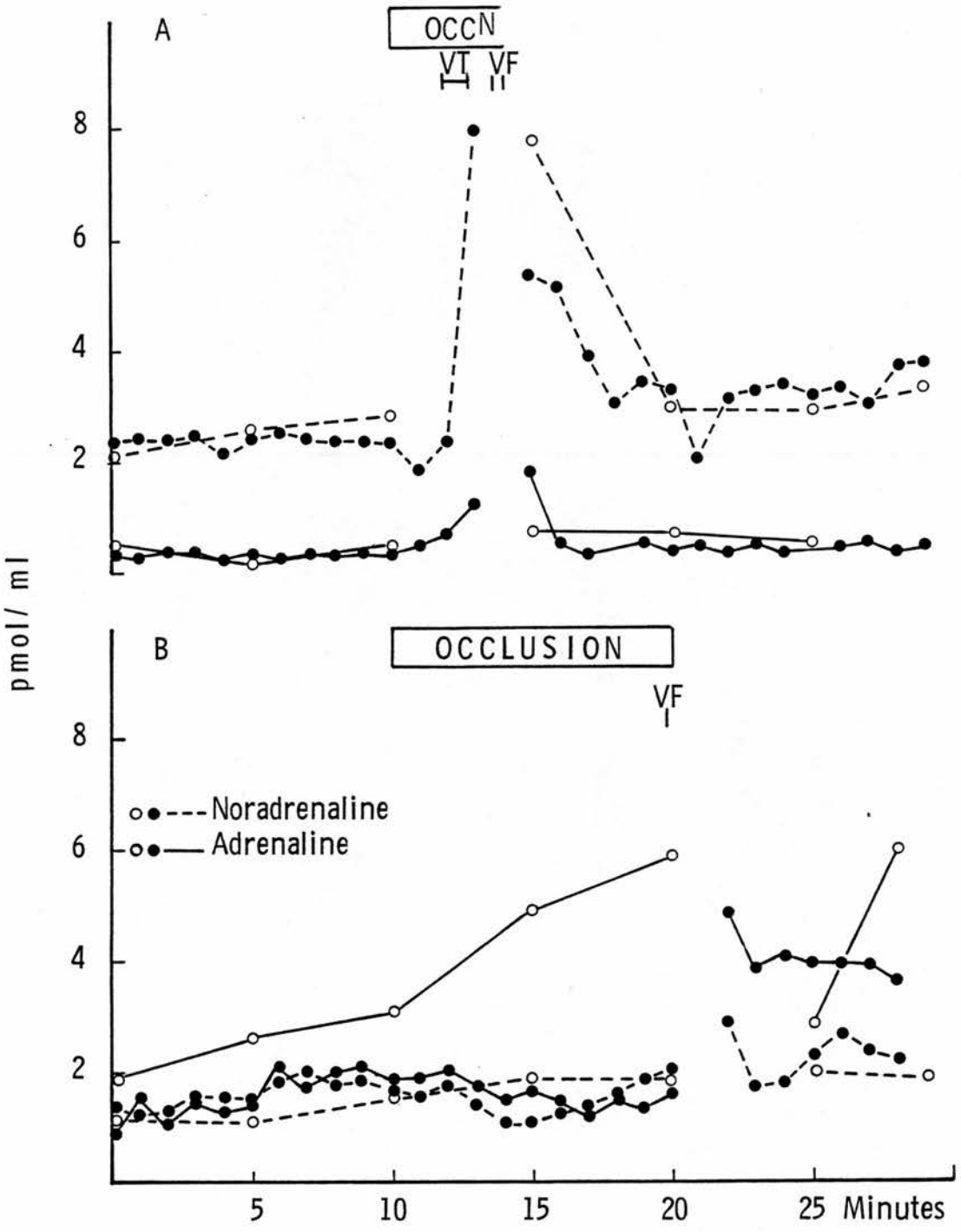


Figure 3.7 Arterial (open circles) and local venous (closed circles) catecholamine responses during spontaneous ventricular fibrillation during coronary occlusion (A) and on reperfusion (B). Vertical lines show defibrillation.

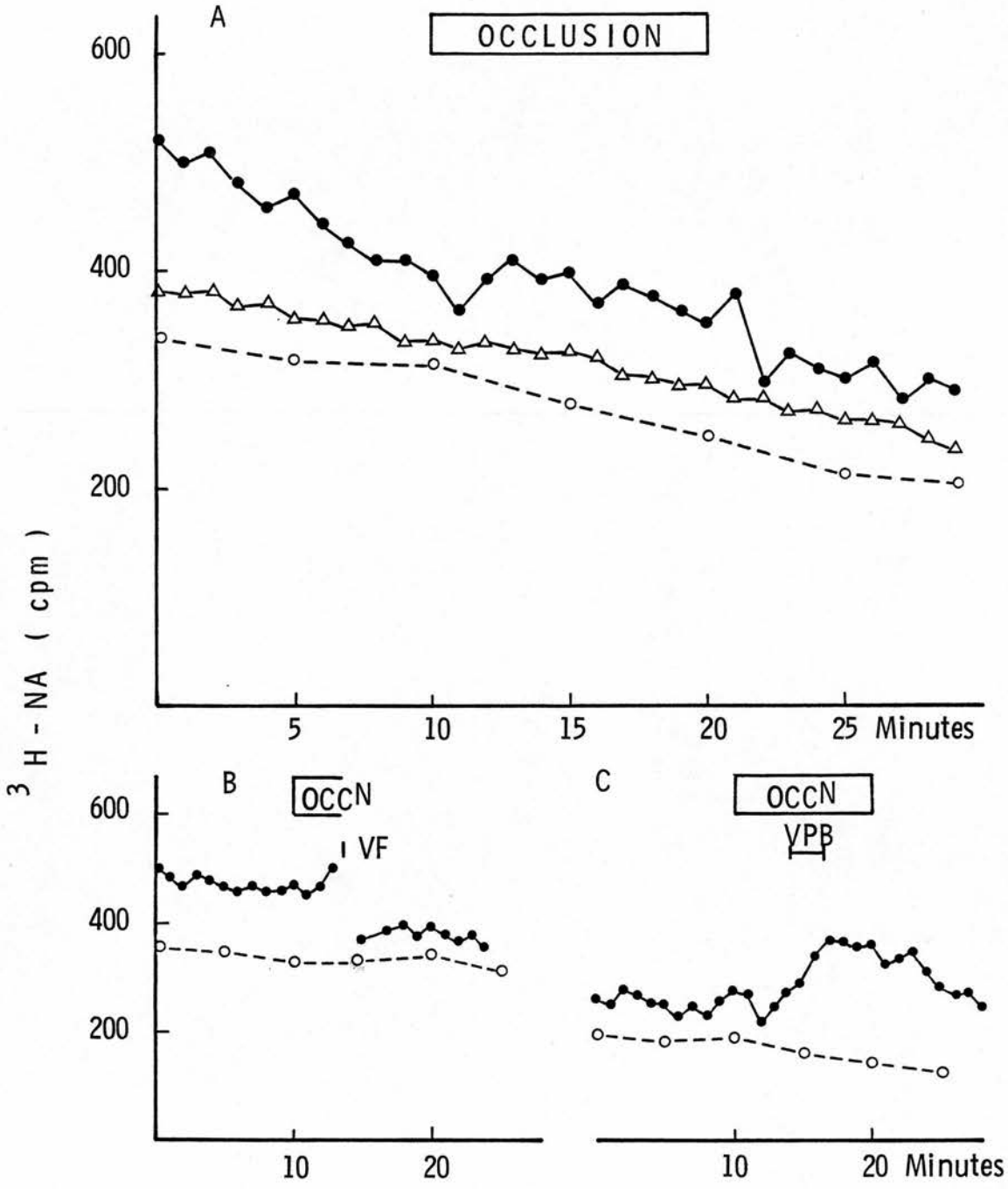


Figure 3.8 ^{3}H-NA in arterial (open circles), local venous (closed circles) and coronary sinus (triangles) effluent. A- mean data (4 experiments). B- ventricular fibrillation. C- ventricular premature beats. B and C from individual experiments.

	BASAL		OCCLUSION					REPERFUSION		
	8'	10'	2'	4'	6'	8'	10'	2'	4'	6'
[NA](pmol/ml)										
Art $\bar{x}$	1.6	1.7	1.6	1.5	2.1	1.7	2.0	1.7	1.9	2.3
SEM	0.2	0.3	0.3	0.3	0.5	0.3	0.4	0.2	0.3	0.9
LV $\bar{x}$	2.1	1.8	2.2	1.9	2.7	2.5	2.4	3.0	2.1	2.4
SEM	0.4	0.4	0.4	0.4	0.7	0.5	0.4	0.4	0.3	0.6
CS $\bar{x}$	1.9	1.8	2.0	2.0	1.6	2.2	1.8	1.9	2.3	2.6
SEM	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.5	0.4	0.3
[A](pmol/ml)										
Art $\bar{x}$	0.6	0.7	0.8	0.7	1.1	1.0	1.1	1.1	1.6	1.4
SEM	0.1	0.2	0.2	0.1	0.2	0.2	0.3	0.3	0.4	0.4
LV $\bar{x}$	0.4	0.3	0.5	0.7	0.7	0.6	0.5	1.2	1.5	1.2
SEM	0.1	0.1	0.2	0.3	0.4	0.3	0.2	0.4	0.3	0.4
CS $\bar{x}$	0.5	0.5	0.5	0.5	0.5	0.8	0.7	0.8	0.7	0.7
SEM	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3

TABLE 3.2: Spontaneous catecholamine concentrations before, during and after LAD occlusion for ten minutes (n = 16; Series II).

	OCCLUSION			REPERFUSION	
	2'	6'	10'	2'	6'
[NA](pmol/ml)					
Art $\bar{x}$	0.1	0.5	0.4	0.2	0.8*
SEM	0.2	0.4	0.3	0.3	0.4
LV $\bar{x}$	0.0	−0.1	0.4	1.0*	0.3
SEM	0.4	0.3	0.4	0.3	0.6
CS $\bar{x}$	0.1	0.1	0.0	0.1	0.7
SEM	0.3	0.4	0.3	0.4	0.4
[A](pmol/ml)					
Art $\bar{x}$	0.2	0.5	0.6*	0.6*	0.8*
SEM	0.3	0.3	0.3	0.3	0.4
LV $\bar{x}$	0.2	0.4	0.1	0.8*	0.8
SEM	0.3	0.4	0.3	0.4	0.5
CS $\bar{x}$	0.0	0.1	0.2	0.3	0.2
SEM	0.3	0.4	0.3	0.3	0.3

* $p < 0.05$ wrt basal level

TABLE 3.2a: Changes in catecholamine concentrations during and after LAD occlusion for ten minutes (n = 16; Series II). Data from Table 3.2.

ischaemia and reperfusion were similar to Series I (Tables 3.2 and 3.2a). Arterial [NA] tended to increase gradually during the sampling period (basal 1.6 $\pm$ 0.2 pmol/ml; reperfusion 2.0 $\pm$ 0.3 pmol/ml; $p < 0.05$) with a more obvious rise in arterial [A] during ischaemia and reperfuson (basal 0.6 $\pm$ 0.2 pmol/ml; ischaemia 0.9 $\pm$ 0.2 pmol/ml; reperfusion 1.4 pmol/ml, $p < 0.05$). Release of small amounts of [NA] from the heart was not significantly modified by regional ischaemia, and although release was maintained from the ischaemic area more than the non-ischaemic area during LAD occlusion (Figure 3.9), this difference did not achieve statistical significance at a 5 per cent level of confidence ($0.05 < p < 0.1$). As before, [NA] release specifically from the previously ichaemic area was readily seen during the first two minutes of reperfusion. Adrenaline extraction across the heart was maintained during the ischaemic period, but was abolished during early reperfusion, indeed minor release of [A] from the previously ischaemic area was evident at this time (Figure 3.9).

No systematic differences between basal catecholamine concentrations (preocclusion) were observed between Series I and II, arterial [A] levels being slightly greater in the morphine-chloralose-urethane series, while basal [NA] levels were greater in the chloralose series (Table 3.1 and 3.2).

As with Series I, spontaneous ventricular ectopic beats (5 experiments) generally did not modify or follow changes in catecholamine release from the heart, although a minor increase in local venous [NA] followed coronary occlusion and ventricular ectopic beats in one experiment. Minute-to-minute sampling of regional effluent indicated whether spontaneous ventricular

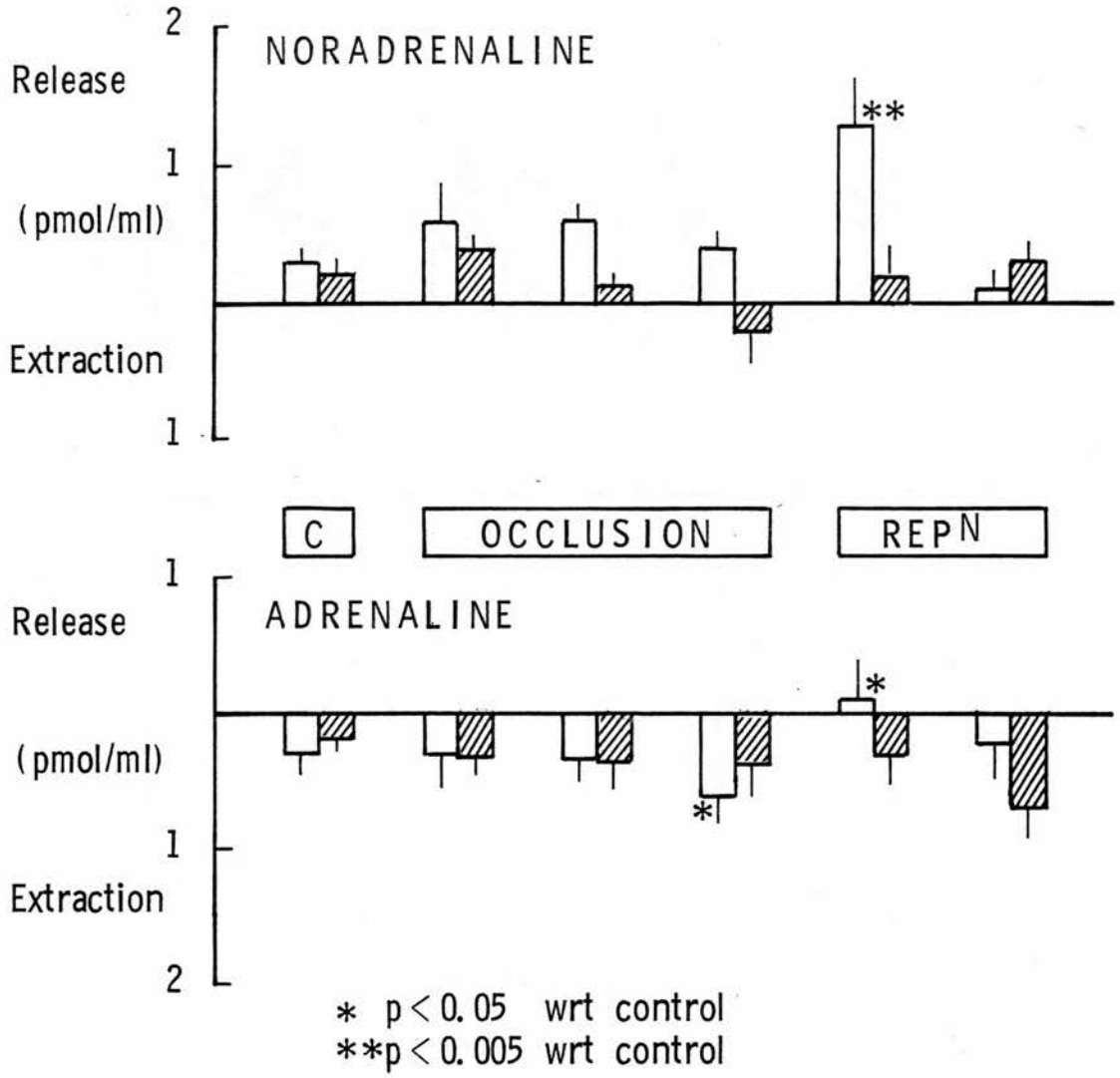


Figure 3.9 Catecholamine release from and extraction across ischaemic (open bars) and non-ischaemic (shaded bars) myocardium before and during coronary occlusion (2, 4-8, 10 min) and on reperfusion (2, 6 min). Data from table 3.2.

	CONTROL	1-NORADRENALINE			CONTROL	TYRAMINE
		5	10	15		5µg/kg/min
		ng/kg/min				
[NA](pmol/ml)						
Art $\bar{x}$	2.7	2.9	2.7	2.7	2.3	4.6
SEM	0.6	0.8	0.6	0.7	0.8	1.6
LV $\bar{x}$	2.4	3.7	4.5^{+}	9.4^{+}	2.9	17.9^{++}
SEM	0.4	1.1	1.2	3.1	1.0	4.5
[A](pmol/ml)						
Art $\bar{x}$	1.0	0.9	1.3	1.6	1.7	2.1
SEM	0.5	0.6	0.5	0.6	0.9	1.0
LV $\bar{x}$	0.4	0.5	0.5	0.5	0.7	1.1
SEM	0.2	0.3	0.2	0.3	0.4	0.4

$^{+}$ $p < 0.05$ $^{++}$ $p < 0.005$ wrt control.

Table 3.3: Arterial and venous catecholamine response to intracoronary l-noradrenaline and tyramine ($n = 6$)

fibrillation followed or resulted in a catecholamine surge. Ventricular fibrillation during LAD occlusion occurred in three experiments. A representative example, corrected for delay in the sampling lines (Figure 3.10), demonstrated that increments in [NA] concentrations at all sampling sites occurred immediately following the arrhythmia rather than before it developed. In this example, no warning tachyarrhythmia developed. It is probable therefore, that generalised sympathetic activation occurred secondary to the loss of effective circulation and/or defibrillation, and was not an antecedent to the arrhythmia.

Validation of detection of catecholamine release

The sensitivity of the model in detecting release of [NA] from the heart was tested in three ways. Firstly, intracoronary infusions of l-noradrenaline at three physiological doses (5, 10, 15 ng/kg/min) was readily detectable in local venous effluent (mean data Table 3.3; example Figure 3.11a). This dose range had no measurable haemodynamic effect. Secondly, intracoronary tyramine (5 µg/kg/min) over 15 minutes resulted in massive myocardial [NA] release (mean data Table 3.3; example Figure 3.11b) and in one study caused spontaneous ventricular fibrillation. Thirdly, stimulation of the left stellate ganglion at 15 minute intervals, for four one-minute periods readily induced cardiac [NA] overflow (Figure 3.12). No significant differences in peak [NA] release were identified although considerable inter-individual variability in [NA] responses was observed.

Lactate Production

Release of lactate into ischaemic venous effluent was observed

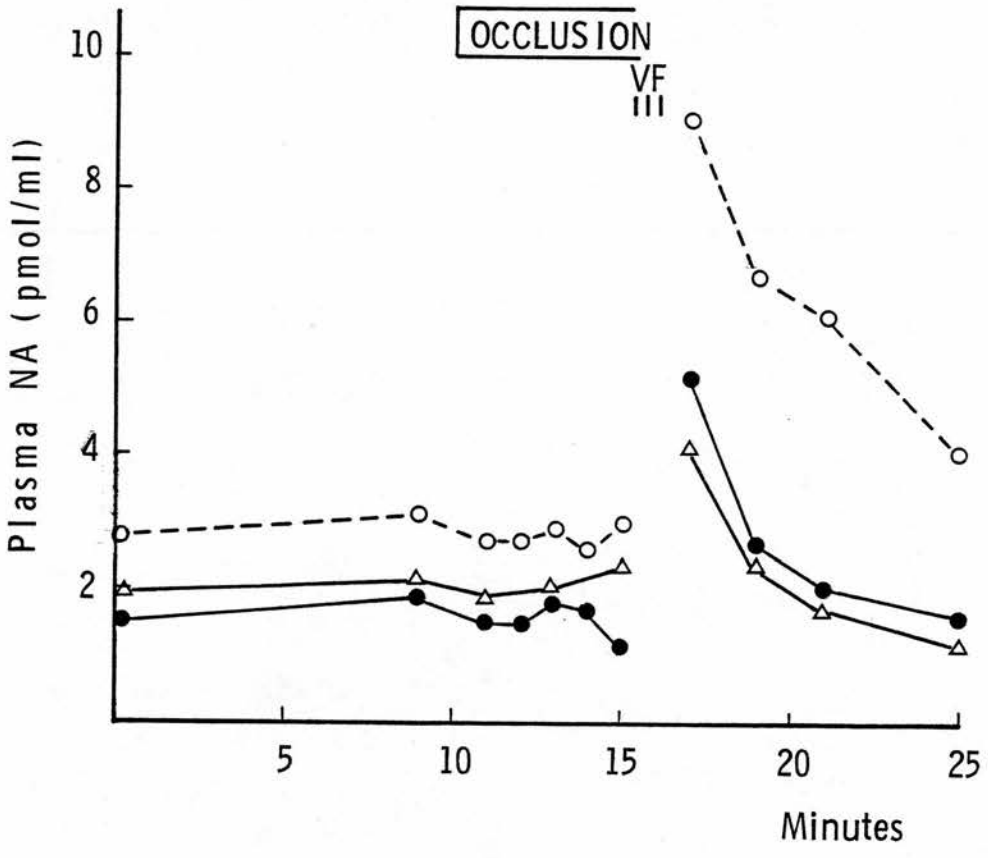


Figure 3. 10 Arterial (open circles), local venous (closed circles) and coronary sinus (triangles) NA before and after spontaneous ventricular fibrillation during coronary occlusion. Vertical lines show defibrillation.

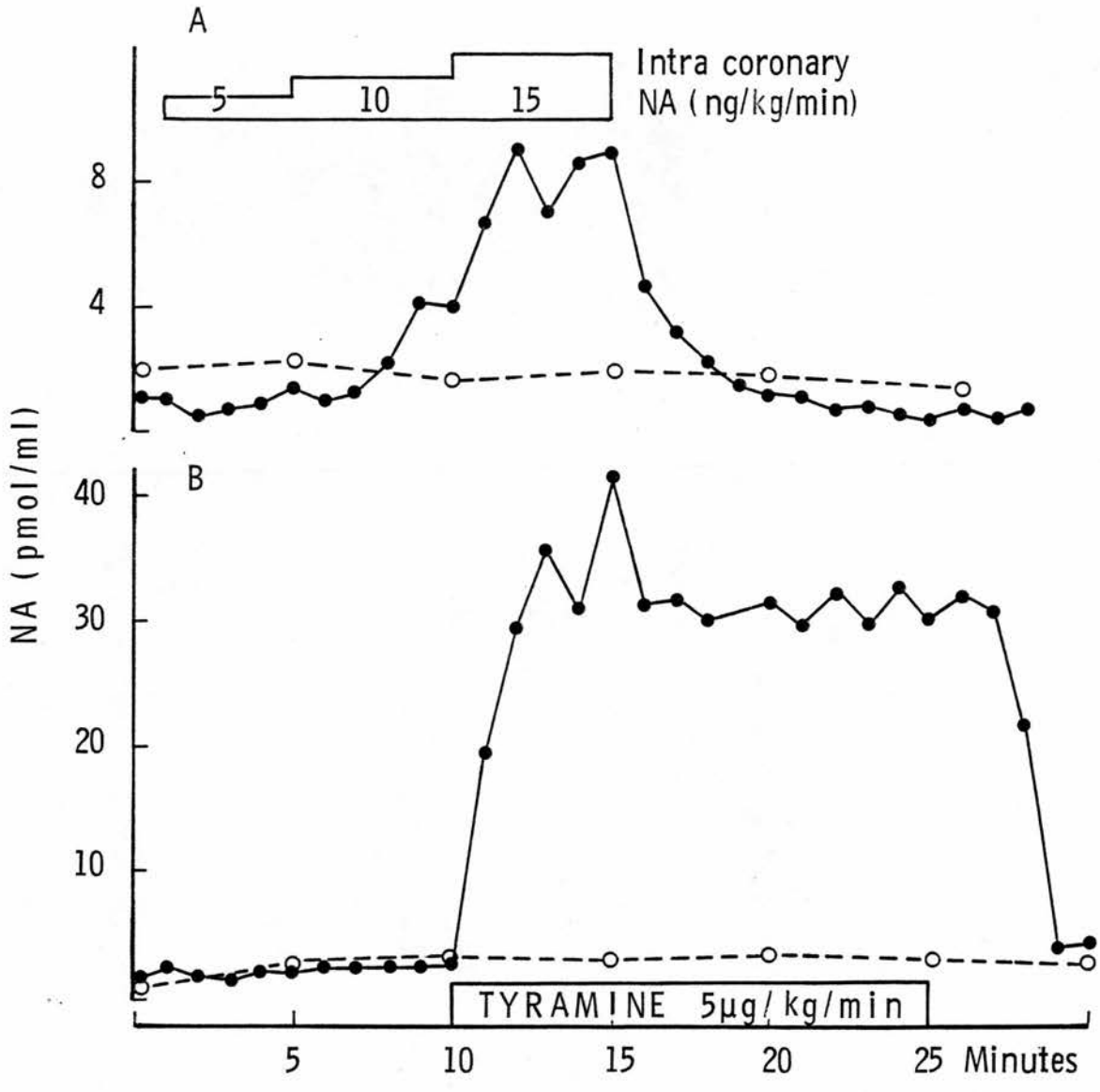


Figure 3.11 Arterial (open circles) and local venous (closed circles) NA responses to intracoronary noradrenaline (A) and tyramine (B).

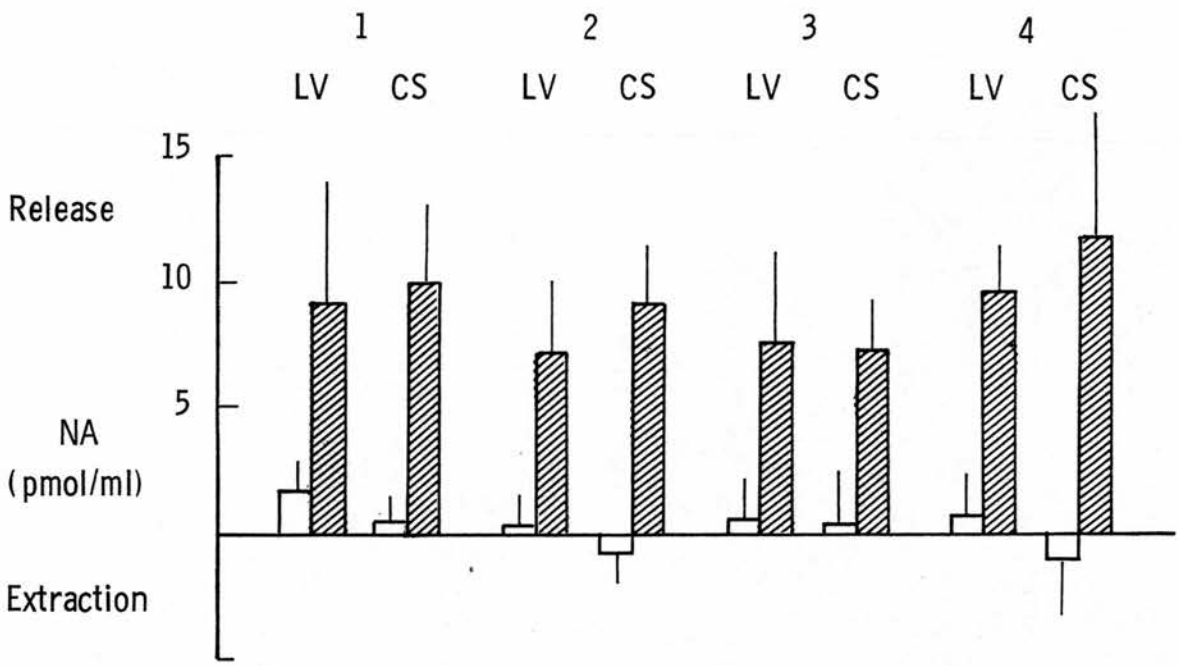


Figure 3.12 Myocardial NA release before (open bars) and immediately after (shaded bars) four one-minute periods of left stellate ganglion stimulation (8v; 4msecs) at 8Hz (n=4).

in all studies with coronary occlusion (mean arteriovenous difference 0.4 ± 0.2 mM/l preocclusion and −4.3 ± 1.2 mM/l during ischaemia: $p < 0.001$; $n = 27$). An example is shown in Figure 3.13. Lactate release was confined to the ischaemic territory, except during reperfusion when some release into the coronary sinus was evident.

Haemodynamic Data

The ischaemic territory (flow less than 50% control) averaged 38 ± 6 per cent of the free left ventricular wall in Series I and 40 ± 5 per cent of the free wall in Series II (p = NS). Mean flow within the ischaemic and non-ischaemic areas for both series is shown in Figure 3.14. Flow reduction to the endocardium (23 ± 4 ml/min/100 g Series I; 22 ± 4 ml/min/100 g Series II) was significantly greater than to the epicardium (33 ± 6 ml/min/100 g Series I; 34 ± 5 ml/min/100 g Series II; $p < 0.025$). Reconstruction of flow maps from each experiment confirmed that the tip of the local venous sampling catheter lay well within the low-flow ischaemic zone.

Changes in mean heart rate and blood pressure during coronary occlusion did not achieve statistical significance in Series I (HR 139 ± 6 beats/min before and 141 ± 7 beats/min during ischaemia; BP 122 ± 9 mmHg before and 120 ± 8 mmHg during ischaemia). In Series II, however, heart rate increased significantly from 149 ± 5 beats/minute before to 154 ± 5 beats/minute during ischaemia ($p < 0.01$). Blood pressure did not change (128 ± 6 mmHg before and 126 ± 6 mmHg during ischaemia).

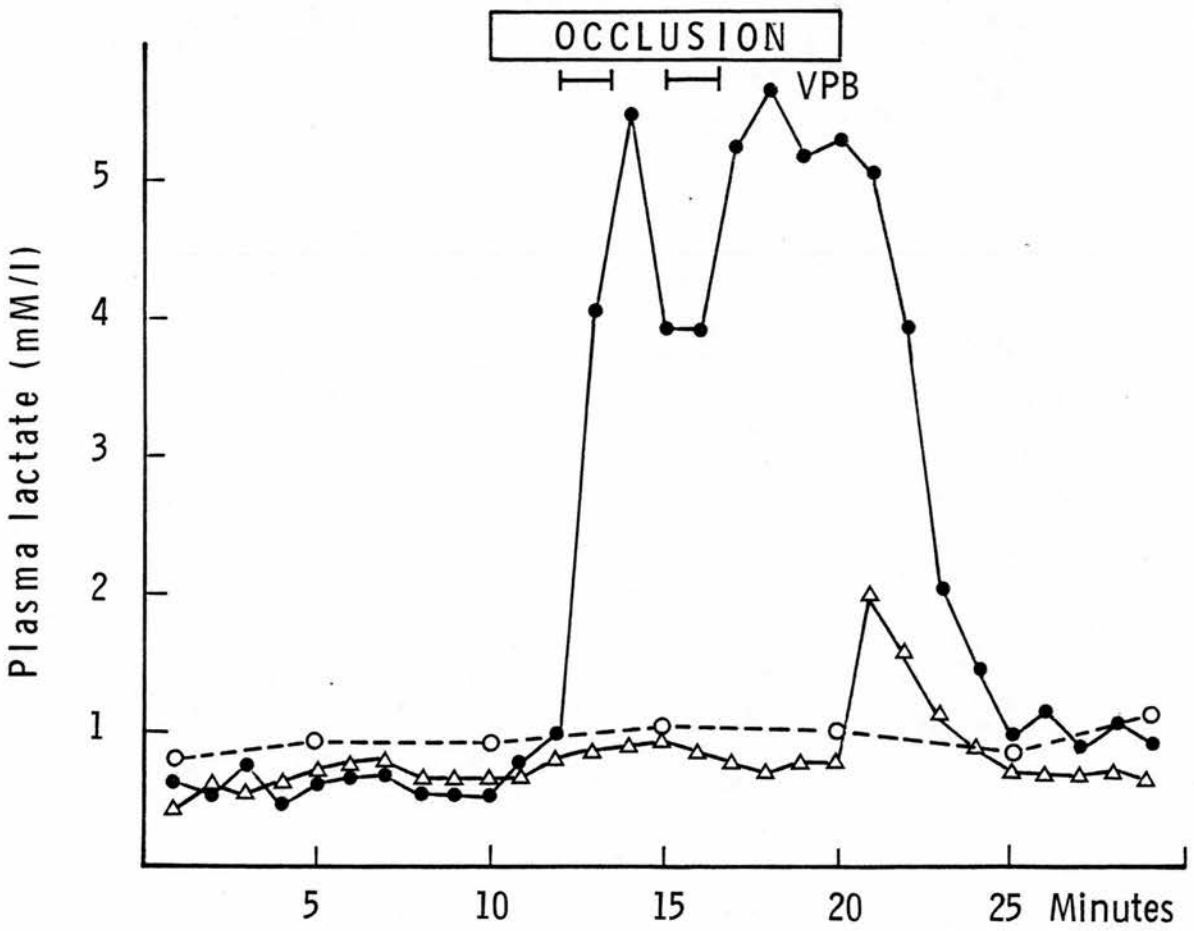


Figure 3.13 Arterial (open circles), local venous (closed circles) and coronary sinus (triangles) lactate during coronary occlusion and reperfusion.

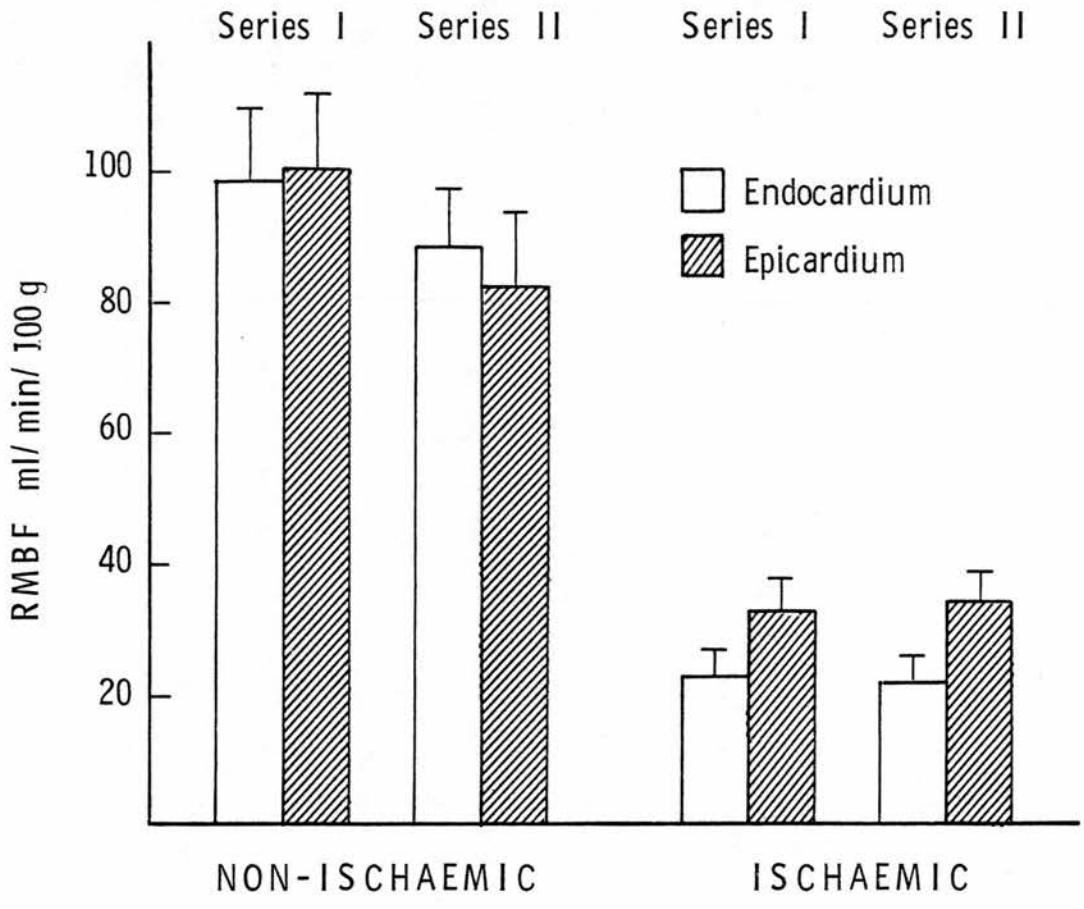


Figure 3.14 Regional myocardial blood flow (RMBF) to normal and ischaemic areas (Series I and II).

DISCUSSION

On theoretical grounds, loss of amine content from infarcting myocardium is readily understood on the basis of known phenomena influencing nerve terminal catecholamine storage. Thus, retention of [NA] within storage vesicles depends on aerobic production of ATP (Bogdanski and Blaszkowski 1975) The amine reuptake pump is linked to the Na^+-K^+-ATPase system, is energy dependent (Wakade and Furchgott, 1968) and is susceptible to inhibition by, for example, increased extracellular potassium or acidosis. Depolarisation of the neuronal membrane inhibits the amine pump and facilitates neurotransmitter diffusion into venous effluent (Lorenz and Vanhoutte, 1975; Verhaeghe et al, 1978). The major intraneuronal degradation enzyme for [NA] , monoamine oxidase, requires intact mitochondrial function (Vanhoutte, 1978), but is not ATP dependent. It is not therefore surprising that myocardial catecholamine levels decline after coronary ligation. The crucial question relevant to the results presented in this chapter is whether these phenomena, applicable to irreversibly damaged tissue, apply equally to the very early arrythmogenic phase associated with reversible ischaemic injury. It is probable that they do not.

Massive release of myocardial catecholamines does <u>not</u> occur within minutes of LAD ligation in the open chest anaesthetised dog. Endogenous [NA] release is readily demonstrable during intracoronary tyramine infusion and cardiac sympathetic nerve stimulation in the absence of coronary occlusion. As the highly sensitive radiochemical method of analysis detects release of physiological quantities of neurotransmitter from the heart, it is therefore most unlikely that massive release from nerve terminals

has been missed even though lymphatic drainage and direct drainage to the ventricular cavity via Thebesian vessels may be enhanced by ischaemia. In this respect, the results stand in sharp contrast to the early studies of Wollenberger and Shahab,(1965), Shahab and Wollenberger,(1967) and Shahab et al,(1969; 1971) in the isolated rat and rabbit heart and in the dog heart in vivo. This group showed loss of an estimated 25 per cent of the total [NA] content of rabbit heart after three to four minutes of arrested perfusion and reperfusion, coinciding with release of lactate from this preparation. Subsequently, in the dog heart, occlusion of the left anterior descending coronary artery for between two and three minutes caused massive release of [NA] (up to 750 ng/min) into the great cardiac vein again coinciding temporally with lactate release at this site. The major part of the release, however, appeared to coincide with the reperfusion rather than the occlusion period. The extent of catecholamine release from the heart after very short periods of ischaemia, reported by Shahab and co-workers, has not been confirmed by several more recent studies, mainly using more sensitive and specific assay methods (Rogg and Bucher 1979; Marshall and Parratt, 1980). In the current study, lactate release during acute ischaemia was maximal during occlusion (Figure 3.13) contrasting with [NA] release that was most easily demonstrated during early reperfusion (Figure 3.3). This suggests that catecholamine release associated with coronary reperfusion is specific to this manoeuvre and not merely washout of catecholamines released during the ischaemic period.

Absence of massive [NA] release during acute ischaemia should not imply absence of enhanced neurosympathetic activity at the nerve terminal over this time. In both studies with ventricular

arrhythmias, prelabelling of the ischaemic territory with ^{3}H-noradrenaline showed a specific increase in counts in ischaemic effluent immediately preceding spontaneous ventricular fibrillation (Figure 3.8b) or during spontaneous ventricular premature beats (Figure 3.8c). This trend for increased release of the parent amine and/or its metabolites, however, did not achieve statistical significance for the grouped results (Figure 3.8a). Release of [NA] into ischaemic effluent was also seen in 2/8 studies with spontaneous ventricular arrhythmias, short of fibrillation. It seems probable, however, that most of the increase in local venous catecholamines with ventricular fibrillation resulted from generalised sympathetic activation at this time, either secondary to circulatory arrest or as a consequence of electrical stimulation of the heart during defibrillation (Blinks, 1966; Euler 1980, 1980a). The rise in [NA] and [A] following defibrillation occurred at all sampling sites (Figure 3.10).

One possible explanation for the apparent absence of major [NA] release from the heart during early ischaemia may lie in enhanced amine reuptake, metabolism and negative feedback operating at the nerve terminal. The powerful reuptake mechanism for example, accounts for the removal of at least two-thirds of released neurotransmitter (Folkow et al, 1967), and was active in the study reported here, evidenced by peak venous [NA] concentrations during steady state intracoronary l-noradrenaline infusion of less than half that predicted by assuming an average LAD flow. With reduced blood flow in the ischaemic area, the diffusion gradient between the extracellular and intravascular space will be diminished and hence removal of neurotransmitter by this mechanism reduced. It is quite possible that reduction in

neurotransmitter diffusion from the synaptic cleft is offset by enhanced neuronal reuptake. An evaluation of the importance of neuronal reuptake and presynaptic negative feedback in controlling release of [NA] from ischaemic myocardium is presented in Chapter 5.

Recent studies into [NA] efflux from the heart during acute ischaemia as opposed to the earlier studies of Wollenberger and Shahab have shown either modest release (Lammerant et al, 1966; Dutta and Booker, 1970; Hirsche et al, 1980) or no release (Marshall and Parratt, 1980; McGrath et al, 1981) of the neurotransmitter at the time of early ventricular arrhythmias. None of these studies as in the present study have related regional catecholamine release to arrhythmias on a minute-to-minute basis. Hirsche et al (1980) noted a small increase (approximately 50%) in coronary sinus [NA] at the time of early ventricular arrhythmias (Phase 1a) but no increments in venous [NA] between 10 and 30 minutes after coronary ligation in the pig. McGrath et al (1981) failed to detect an increase in coronary sinus [NA] during LAD ligation in five anaesthetised dogs, although both the site of occlusion and coronary venous lactate concentrations during occlusion in this study suggested a small area of mild ischaemia.

The increase in arterial [A] during coronary occlusion is in keeping with the studies of Ceremuzynski and co-workers from the late 1960's and early 1970's, showing enhanced [A] excretion from the adrenal medulla within minutes of coronary occlusion on the basis of a reflex originating from the ischaemic area. Increased [A] extraction selectively across the ischaemic area in the experiments reported here was an unexpected finding and supports

the view that uptake processes are active in the ischaemic area at this time. Extraction was rapidly abolished for several minutes by coronary reperfusion (Figure 3.5 and 3.9). One explanation for these findings is that flow is a major determinant of [A] uptake by the heart. Low flow (ischaemia) increases uptake, provided of course that uptake (neuronal and extraneuronal) mechanisms are intact, whereas high flow (reactive hyperaemia) reduces uptake. Reperfusion-induced release of [A] extracted during the immediately preceding ischaemia, may also have contributed to the rise in local venous [A] at this time.

The importance of reperfusion-induced [NA] release in the genesis of reperfusion arrhythmias has not been widely investigated. Indeed, until the recent demonstration of the potential importance of vasospasm in the genesis of reversible and irreversible ischaemic injury in man (Berndt et al, 1977; Oliva and Breckenridge, 1977; Maseri et al, 1978), reperfusion had not been considered as a likely mechanism of arrhythmogenesis. Taking flow into account, the observed small reperfusion-induced overflow of [NA] from the previously ischaemic area represents a several-fold increase in [NA] output, sustained for a maximum of two minutes.

Although reperfusion-induced [NA] overflow was noted only during the first two minutes, [NA] output from the previously ischaemic area may have persisted for a longer period because of the dilutional effect of reactive hyperaemia on the [NA] concentration in local venous effluent.

reperfusion arrhythmias have suggested that instantaneous arrhythmias occurring within the first minute of reperfusion are

associated with heterogeneous recovery and fractionation of activation across the heart, rather similar to (and correlated with) the antecedent period of arrhythmias during coronary ligation (Levites et al, 1975; Kaplinsky et al, 1981). In contrast, the second less common phase of delayed ventricular arrhythmias (rarely progressing to fibrillation) occuring two to seven minutes after coronary reperfusion develops when electrical activity has become synchronous (Kaplinsky et al, 1981) and enhanced [NA] release from the heart has subsided. The cause of [NA] release on reperfusion is speculative. Increased transmembrane calcium flux, certainly important in reperfusion-induced cellular injury in myocytes (Walen et al, 1974) may result in calcium overload of the neuroplasm and excessive exocytotic release of stored [NA]. Alternatively, enhanced local [A] concentrations may act as promotors of [NA] release through activation of presynaptic beta receptors (Langer, 1978). Adrenergic mechanisms have been implicated in reperfusion arrhythmias, alpha- but not beta-adrenoceptor antagonists being effective in their suppression (Corr and Gillis 1978; Sheridan et al, 1980).

Catecholamine fluorescence of ischaemic and infarcting myocardium has not shown consistency with regard to the rate of decrease in nerve terminal fluorescence after coronary ligation. Paessens and Borchard (1980) showed some reduction in fluorescence two to four hours after coronary occlusion with areas completely devoid of fluorescence only after 12-24 hours. Abrahamsson et al (1982) showed reductions in fluorescence in the central ischaemic area of rat heart after 2 1/2 hours. At 30 minutes, small areas of reduced fluorescence were visible but the [NA] content of sham-operated animals was also diminished at this time suggesting a

non-specific effect. A similar time course of depletion over some hours has been reported in the dog (Vasku et al, 1978). In contrast, Hirsche et al (1981) have suggested extremely rapid reductions in nerve terminal catecholamine content after LAD occlusion in the pig model, substantial reductions in fluorescence being detected after three to four minutes of ischaemia. This depletion was readily prevented by lignocaine and prostacyclin. The reasons for such variability in results is unclear but species differences and differences in the processing of ischaemic tissue may be important. The influence of acidosis, hyperkalaemia and other metabolic changes typical of ischaemia on the technique of fluorescence histochemistry has been poorly defined.

No biologically important differences between the effects of morphine/chloralose/urethane and chloralose anaesthesia on catecholamine responses were oberved. The slightly increased heart rate and blood pressure with chloralose alone, and the small further increase in heart rate after coronary occlusion with this agent, probably reflect absence of the vagotonic influence of morphine (De Silva et al, 1978a). Activation of parasympathetic activity antagonises cardiac sympathetic responses directly and through inhibition of nerve terminal [NA] release via a peripheral muscarinic mechanism (Lavallee et al, 1978, 1980). This may also explain the somewhat lower basal [NA] levels in arterial and venous plasma in Series I (with morphine) compared to Series II (chloralose alone). Ventricular arrhythmias tended to occur more frequently in Series II (50 per cent) than in Series I (36 per cent) but differences did not achieve statistical significance.

4 TIME-DEPENDENCY OF NEUROSYMPATHETIC RESPONSIVENESS: RELATIONSHIP TO EARLY ARRHYTHMIAS

The purpose of this study was to assess regional catecholamine release from the heart and electrophysiological responses during direct stimulation of efferent cardiac sympathetic nerves at intervals after coronary occlusion. Over the past two decades, there has been an explosion of interest and information concerning factors that modulate peripheral release of sympathetic neurotransmitter at the nerve terminal (Shepherd and Vanhoutte, 1981). Much of the work, however, has been in vitro and in tissues other than the heart. It is possible, nonetheless, that neurohumoral or metabolic changes in ischaemic myocardium might, at least in the short term, inhibit or depress nerve terminal responses to enhanced efferent neural discharge and hence explain absence of major catecholamine release from the heart (Chapter 3).

A moderate degree of acidosis (for example a reduction in pH from 7.4 to 7.1) is without effect on the basal tension or responsiveness of isolated cutaneous veins to increasing concentrations of [NA] , but does depress the response to sympathetic stimulation (Tobian et al, 1959; Vanhoutte et al, 1968), possibly by reducing calcium entry into the nerve terminal and inhibiting exocytotic release of neurotransmitter (Verbeuren et al, 1978). An increase in local potassium concentration can have at least three actions on the nerve terminal. At concentrations below 20 mmol/l, inhibition of [NA] release in response to nerve stimulation is observed, but neuronal reuptake is also reduced. With higher concentrations, the neurotransmitter is liberated directly (Kirpekar et al, 1972; Lorenz and Vanhoutte, 1975). Similarly, adenosine, adenine nucleotides, histamine and 5-hydroxtryptamine have all been shown to inhibit [NA] release in response to nerve stimulation (Verhaeghe et al, 1977; McGrath and

Shepherd, 1978; De Mey et al, 1979). Activation of presynaptic alpha adrenoceptors may limit [NA] release via a local negative feedback loop (Langer,1978; Starke,1978), an aspect discussed further in Chapter 5. Acetylcholine release from vagal efferents may also have an inhibitory effect (Vanhoutte,1974). Arachidonic acid metabolites synthesised in the heart and released during acute ischaemia may attenuate adrenergic transmitter release (Wennmalm, 1978; Khan and Malik, 1982) while peptides such as angiotensin II may have a facilitatory role (Lanier and Malik, 1983).

Stimulation of the ansa subclavia from the right and left stellate ganglion in the dog activates most of the efferent sympathetic nerves to the heart. Nerves from the left side provide the major sympathetic input to the left ventricle. Noradrenaline overflow into the coronary sinus and contractile responses of the left ventricle are significantly greater with left compared to right sided stimulation. Chronotropic responses, however, are much less, reflecting the major sympathetic input to the sinus node from the cardiac stellate nerve, a branch from the right stellate ganglion (Levy and Blattberg, 1977). It has recently been suggested that heart rate per se may influence the dissipation of released neurotransmitter into venous effluent, especially if reuptake processes are impaired (Levy,1982). Cardiac responses to prolonged trains of sympathetic stimuli are maximal and remain constant over 1-2 minutes but thereafter decline at high rates of stimulaton (Levy and Blattberg, 1976). Left stellate ganglion stimulation for one-minute periods at both low (1 Hz) and high (10 Hz) frequency was therefore selected for these investigations.

METHODS: PROTOCOLS

Studies were performed on adult mongrel dogs (10 - 25 kg) of either sex anaesthetised with pentobarbitone (25 mg/kg intravenously for induction; 4 mg/kg/hr for maintenance) and ventilated on room air using a Harvard respirator. After placement of infusion and sampling catheters in both femoral vessels, and an aortic catheter for monitoring blood pressure, a left lateral thoracotomy was performed, the left fifth rib resected and the heart suspended in a pericardial cradle. After lung retraction, good visualisation of the left stellate ganglion was achieved. The anterior and posterior ansa subclavia from this ganglion were carefully dissected free from pleura, fat and subcutaneous tissue and stimulated using a bipolar silver hook electrode attached to a Grass Model S48 stimulator. Square wave pulses (4 ms duration) at supramaximal voltage (8 - 10 v) and varying frequency (1 - 20 Hz) were used. After a test stimulation over 30 seconds at 10 Hz to confirm an appropriate inotropic and chronotropic response - usually a 20 - 40 per cent increase in mean blood pressure, widened pulse pressure and increase in heart rate of 10 - 20 beats/minute, associated in 70 per cent of studies with the development of nodal rhythm - the heart was paced continuously from the left atrium throughout each experiment at a rate of 5 - 10 beats/minute faster than the peak chronotropic response observed. The paced rate varied overall between 150 and 192 beats/minute in different experiments but was kept constant for each study.

The left anterior descending coronary artery (LAD) was isolated within 2 cms of its origin distal to the first septal perforator for coronary occlusion using a Mayfield occlusion clip.

As described in Chapter 3, venous catheters were inserted retrogradely into the centre of the LAD territory and antegradely on the posterolateral surface of the heart, draining towards the coronary sinus. The left atrium was catheterised via the appendage and the animal heparinised (100 u/kg) after completion of surgery.

At least 30 minutes elapsed between completion of surgery and sample collection. In control studies without ischaemia, 1 ml samples were taken simultaneously over 15 seconds from arterial and both cardiac venous sampling sites at minute intervals for two minutes before, after and during the final 15 seconds of one minute periods of ansa stimulation at varying frequency.

In Group I, minute-to-minute changes in catecholamine and lactate levels at arterial and both cardiac venous sampling sites were measured as above, before, during and after ansa stimulation for one minute at 1 and 10 Hz in the absence of ischaemia and at either low or high frequency during two successive ten minute periods of LAD occlusion followed by reperfusion (Figure 4.1a). Stimulation was commenced exactly four minutes after coronary occlusion in each period to allow sympathetic activation during the phase of maximum liklihood of spontaneous arrhythmias. At least 15 minutes recovery was allowed after the control ansa stimulation before ischaemia and at least one hour recovery was allowed between the first and second occlusion. Regional myocardial blood flow was measured by microsphere injection three minutes after the second occlusion and again one minute after reperfusion. Ventricular fibrillation was managed by internal DC defibrillation after removal of the coronary occlusion clip. In view of the changes in catecholamines resulting from ventricular fibrillation (described

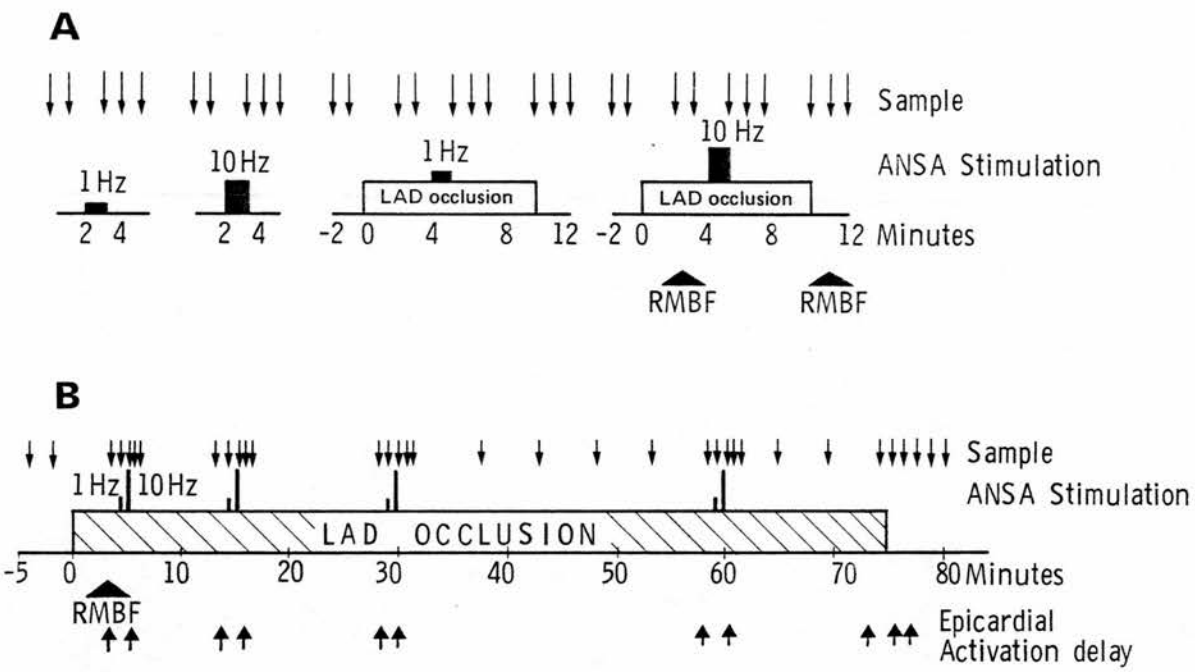


Figure 4.1 Experimental protocols. Time-dependency of neurosympathetic responsiveness. In Group 1 (A), during two ten-minute periods of LAD occlusion, the ansa was stimulated for one minute at 1 and 10 Hz respectively before and four minutes after occlusion. Minute-to-minute changes in catecholamines in ischaemic (I) and non-ischaemic (NI) effluent were assessed (arrows). In Group 11 (B), activation delay and catecholamine overflow were assessed during a single 75 minute period of LAD occlusion and reperfusion. Sympathetic stimulation was undertaken at 5, 17, 30 and 60 minutes after the onset of ischaemia.

in Chapter 3), all samples collected after this arrhythmia were excluded from the analysis. The anterior local venous catheter was positioned within the centre of the LAD perfusion territory, thus draining venous blood from predominantly ischaemic myocardium (I) during coronary occlusion. The posterolateral venous catheter towards the coronary sinus drained non-ischaemic myocardium (NI) from the area of perfusion of the circumflex coronary artery.

In Group II, LAD occlusion was continued for 75 minutes before reperfusion and myocardial catecholamine and lactate overflow from ischaemic and non-ischaemic areas assessed during four successive periods of low and high frequency ansa stimulation: twice at the times of peak incidence of spontaneous arrhythmias 5 and 17 minutes after occlusion (Harris phase 1a and 1b respectively), and twice when arrhythmias are relatively rare 30 and 60 minutes after occlusion (Figure 4.1b). Ventricular fibrillation was documented in three experiments during ansa stimulation at 5 minutes and once during stimulation at 17 minutes. Arrhythmias were not observed during stimulation at 30 or 60 minutes. Defibrillation without removal of the coronary occlusion clip restored sinus rhythm in two out of the three episodes of fibrillation at 5 minutes and in the episode at 17 minutes. As the studies in Chapter 3 showed that catecholamine levels return to pre-fibrillation values within five minutes of the restoration of stable sinus rhythm, data from these experiments was included in the overall analysis, with the exception of the first five minutes after defibrillation. Regional myocardial blood flow was assessed three minutes after occlusion. Epicardial activation delay across the ischaemic region was mapped immediately before ansa stimulation, immediately after each one-minute period of high frequency stimulation and one and three

minutes after coronary reperfusion (Figure 4.1b). Mean activation delay in the ischaemic area was derived from electrodes showing local activation times equal to or greater than 40 msecs at any period after coronary occlusion.

All samples were collected into precooled tubes ($4^{o}C$) containing heparin and sodium metabisulphite. Plasma was immediately separated and frozen for subsequent analysis as described previously.

Changes in peak myocardial catecholamine and lactate overflow and blood pressure following single periods of ansa stimulation and coronary reperfusion were analysed by comparing levels immediately before with those during nerve stimulation or reperfusion using Student's t-test for pair differences. Trends in myocardial catecholamine overflow during sequential periods of ansa stimulation and with time were evaluated by analysis of variance and computed modified t-statistic. Non-parametric ranking methods (Wilcoxon test for pair differences) were used for analysis of epicardial activation delays before and after nerve stimulation. Data are expressed as mean $\pm$ standard error of the mean with a five per cent level of confidence considered statistically significant.

RESULTS

In the non-ischaemic state, stimulation of the ansa for one minute resulted in frequency-dependent release of [NA] into both anterior and lateral cardiac veins with peak release between 10 and 20 Hz (Figure 4.2). Release was greater from the lateral circumflex territory than the anterior LAD territory (overflow at

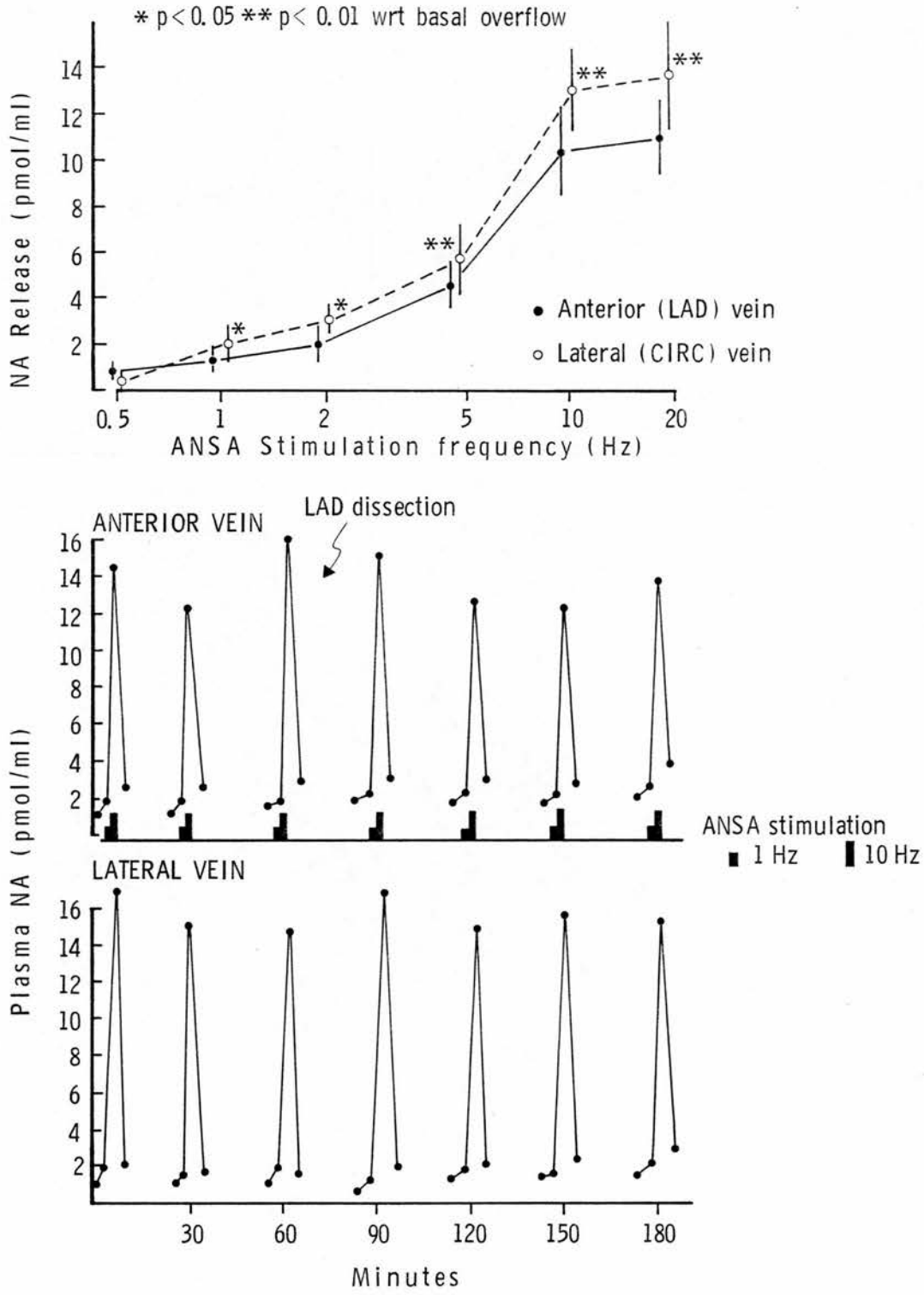


Figure 4.2 Frequency - response curves (semi-log scale) and reproducibility of regional myocardial NA release in response to sympathetic stimulation. Release was greater from the lateral compared to the anterior surface of the heart (n=7, upper panel). Repeated ansa stimulation showed no evidence of tachyphylaxis or reduction in overflow with LAD dissection during a three hour period.

10 Hz 13.4 ± 1.9 pmol/ml circumflex; 10.2 ± 2.0 pmol/ml LAD: $p < 0.05$), possibly reflecting different densities of sympathetic innervation from the left stellate ganglion in these areas.

For subsequent experiments, as indicated above 1 and 10 Hz stimulation was selected for analysis of the catecholamine response. Repeated stimulation of the ansa at these frequencies on seven successive occasions resulted in reproducible myocardial [NA] release from both sites with no evidence of tachyphylaxis over a three hour period (Figure 4.2). When dissection of the LAD (without occlusion) was carried out after the third period of stimulation this did not modify peak [NA] relase during four subsequent stimulations, suggesting that the dissection itself did not impair the functional responsiveness of the sympathetic nerves over this time.

Group I- Effect of ischaemia on stimulation-induced [NA] release

The pattern of [NA] response to ansa stimulation was not significantly modified by two ten-minute periods of LAD occlusion in seven experiments (Group I). Arterial [NA] increased gradually over the periods of ischaemia (basal [NA] 1.7 ± 0.4 pmol/ml; [NA] after second occlusion 3.3 ± 0.7 pmol/ml {$p < 0.05$}), but [NA] release from I and NI areas was not significantly different from that prior to ischaemia (Table 4.1). Peak release at 1 Hz from the anterior local vein was 1.1 ± 0.8 pmol/ml basal and 2.2 ± 1.9 pmol/ml during ischaemia; at 10 Hz release was 7.5 ± 4.3 pmol/ml basal and 7.8 ± 5.0 pmol/ml during ischaemia, p = NS. Similarly, [NA] release from the non-ischaemic lateral surface of the heart was not modified by LAD occlusion (Table 4.1). Myocardial [NA]

		BASAL			FIRST OCCLUSION		SECOND OCCLUSION		REPERFUSION	
		Cont	1Hz	10Hz	2'	1Hz	2'	10Hz	1'	3'
[NA](pmol/ml)										
Art	$\bar{x}$	1.7	1.7	2.4	2.0	2.5	3.2	3.3	3.9	3.1
	SEM	0.4	0.4	0.6	0.6	0.7	0.6	0.7	1.6	1.4
LV	$\bar{x}$	2.0	2.8	9.9	3.4	4.7	3.1	11.0	5.1	3.9
	SEM	0.4	0.4	3.5	1.0	1.8	0.6	3.8	1.9	1.7
CS	$\bar{x}$	2.5	4.1	13.8	2.9	3.9	3.6	16.5	3.0	2.8
	SEM	0.4	0.5	5.4	0.8	0.7	0.9	5.0	2.0	1.9
[A](pmol/ml)										
Art	$\bar{x}$	0.6	0.7	0.5	0.6	0.7	1.8	3.0	5.7	4.7
	SEM	0.3	0.4	0.3	0.3	0.5	1.1	2.3	4.8	3.7
LV	$\bar{x}$	0.2	0.4	1.1	0.4	0.4	0.5	1.2	4.1	3.7
	SEM	0.1	0.1	0.7	0.1	0.1	0.3	0.7	3.5	3.1
CS	$\bar{x}$	0.2	0.4	0.7	0.3	0.3	0.4	0.8	1.7	1.8
	SEM	0.1	0.2	0.4	0.1	0.1	0.1	0.4	1.2	1.5

Table 4.1: Myocardial catecholamine responses to low and high frequency sympathetic nerve stimulation before and during coronary occlusion and to reperfusion (Group I, n = 7). Reperfusion data combined from first and second occlusions.

release from ischaemic and non-ischaemic territories is shown in Figure 4.3.

Arterial [A] also increased progressively during the two periods of ischaemia with further increase on reperfusion (Table 4.1). Myocardial [A] extraction was maintained during occlusion, ansa stimulation and reperfusion although extraction was significantly less and output of [A] (derived from mean endocardial and epicardial blood flows shown in Table 4.2) significantly greater from I compared to NI during reperfusion ([A] extraction across I 28 $\pm$ 8 per cent and output 11.0 $\pm$ 6.1 pmol/min/g one minute after reperfusion: [A] extraction across NI 70 $\pm$ 18 per cent and output 1.3 $\pm$ 2.1 pmol/min/g at the same time: $p < 0.01$).

The ischaemic area (flow < 50 per cent of normal myocardium) involved 27 $\pm$ 4 per cent of the epicardium and 31 $\pm$ 4 per cent of the endocardium of the free left ventricular wall. Lactate production was restricted to the ischaemic area where mean blood flow decreased to 30 per cent and 18 per cent of flow in the non-ischaemic epicardium and endocardium respectively (Table 4.2).

Reperfusion induced a small but significant spontaneous release of [NA] from the ischaemic area (1.2 $\pm$ 1.1 pmol/ml, Table 4.1 and Figure 4.3). This represented an approximately 30 fold increase in mean [NA] output from I (from 0.5 $\pm$ 0.4 pmol/min/g four minutes after occlusion to 16.2 $\pm$ 3.6 pmol/min/g one minute after reperfusion) while output from non-ischaemic myocardium was unchanged (2.6 $\pm$ 1.3 pmol/min/g during occlusion; 2.3 $\pm$ 1.8 pmol/min/g during reperfusion).

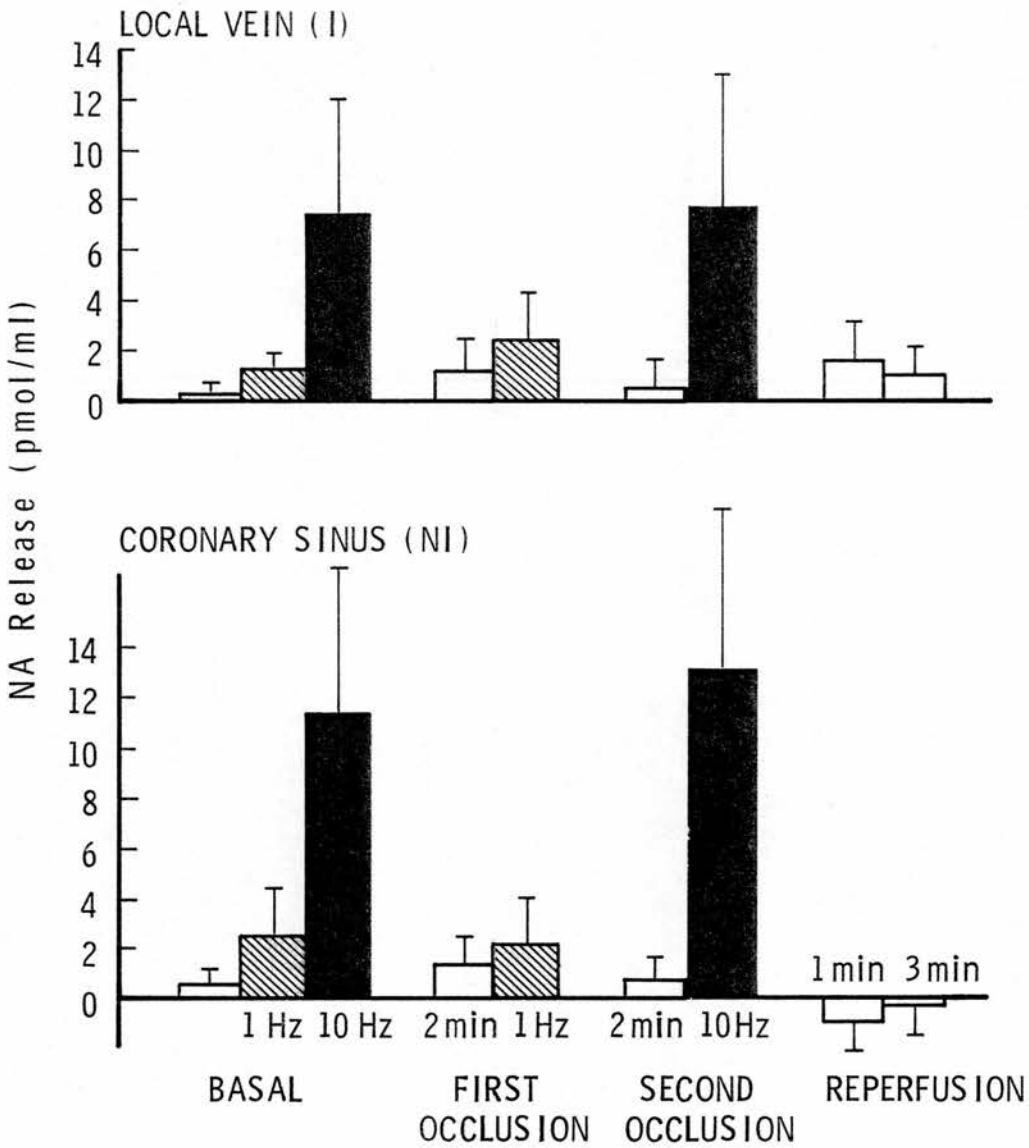


Figure 4.3 Noradrenaline release from I and NI myocardium with ansa stimulation before and during LAD occlusion and on reperfusion. Data from Table 4.1.

		OCCLUSION		REPERFUSION	
		ISCHAEMIC	NON-ISCHAEMIC	ISCHAEMIC	NON-ISCHAEMIC
Regional myocardial blood flow (ml/min/100 g)					
EPICARDIUM	$\bar{x}$	20	67	219	69
	SEM	6	9	32	5
ENDOCARDIUM	$\bar{x}$	14	77	316	86
	SEM	4	9	42	13
Myocardial lactate extraction (%)					
	$\bar{x}$	-260	18	-104	9
	SEM	94	11	78	23

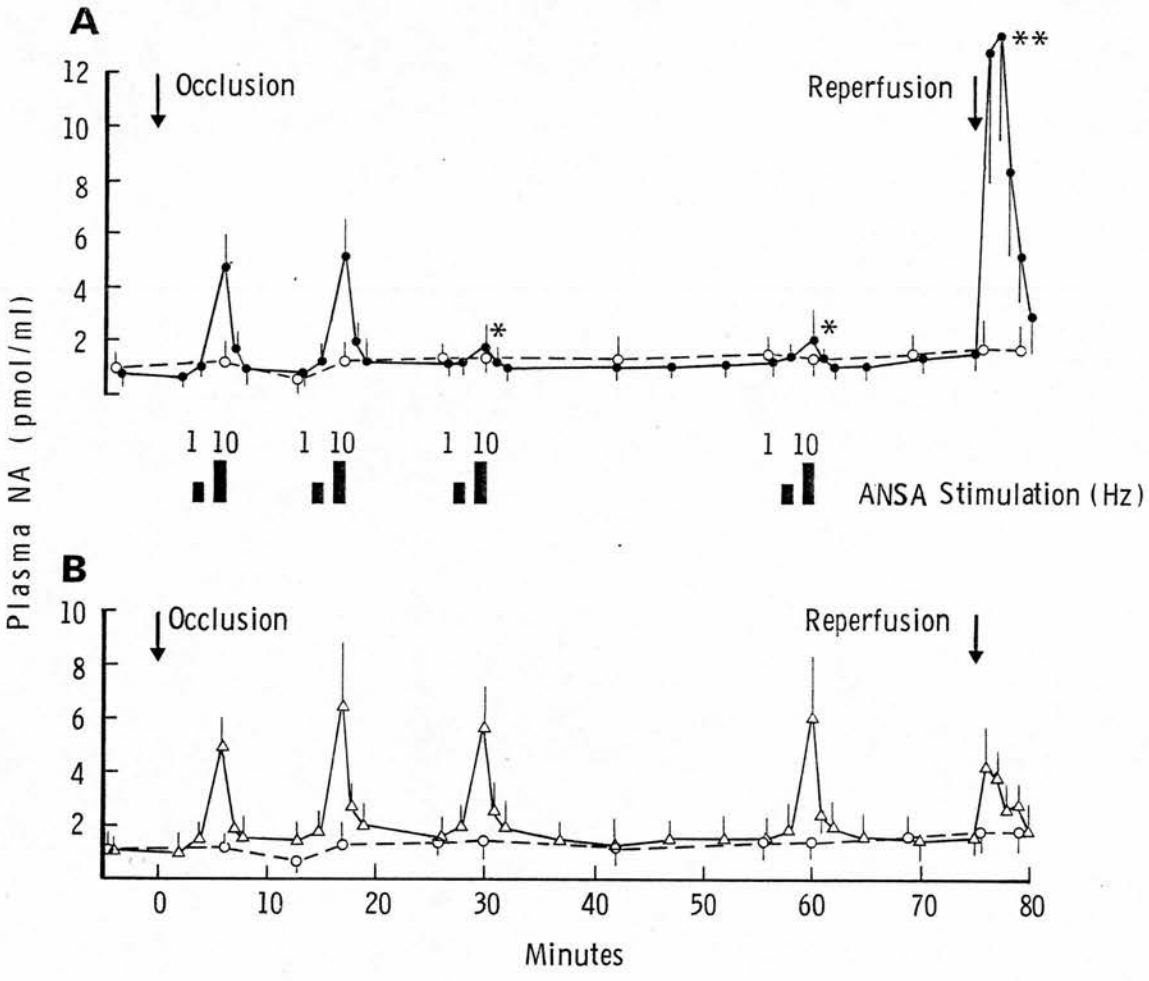
Table 4.2: Regional blood flow and lactate extraction across ischaemic and non-ischaemic myocardium during 10 minutes LAD occlusion and one minute after reperfusion (Group I, n = 7).

Group II- Effect of ischaemia duration on stimulation-induced [NA] release

In contrast to responses during a ten minute period of ischaemia, catecholamine release and changes in epicardial activation delay varied with ansa stimulation at intervals during 75 minutes of ischaemia. Peak [NA] in I and NI effluent was similar 5 and 17 minutes after LAD occlusion but levels were significantly less in I at 30 and 60 minutes (Figure 4.4a), corresponding with a reduction in [NA] release of approximately eighty per cent from this area (Figure 4.5a). [NA] responses from non-ischaemic effluent (Figure 4.4b) and [NA] release from this area (Figure 4.5b) were not modified by coronary occlusion. Reperfusion induced substantial release of [NA] from the previously ischaemic area and lesser release from non-ischaemic tissue (Figure 4.4).

As shown in Figure 4.6, a progressive increase in arterial [A] was maintained during the 75 minute occlusion and on reperfusion. [A] increased from 1.0 $\pm$ 0.3 pmol/ml prior to occlusion to 1.9 $\pm$ 0.5 pmol/ml during ischaemia ($p < 0.05$) with a further increase to 2.8 $\pm$ 0.8 pmol/ml during the first five minutes of reperfusion. Although levels of [A] in venous effluent remained constant during occlusion, extraction across I increased from 0.7 $\pm$ 0.2 to 1.5 $\pm$ 0.6 pmol/ml ($p < 0.02$). Extraction across I, however, decreased significantly (1.0 $\pm$ 0.5 pmol/ml; $p < 0.05$) during reperfusion.

Changes in epicardial activation delay within the ischaemic area before and after ansa stimulation (Table 4.3) showed significant differences dependent on the duration of coronary occlusion. Stimulation after five minutes of ischaemia increased



* $p < 0.05$ wrt responses at 5 and 17 min
** $p < 0.01$ wrt pre-reperfusion NA

Figure 4.4 Noradrenaline responses in arterial (open circles), local venous (closed circles, A) and coronary sinus (triangles, B) plasma with ansa stimulation 5, 17, 30 and 60 minutes after LAD occlusion and on reperfusion (Group 11, n= 9).

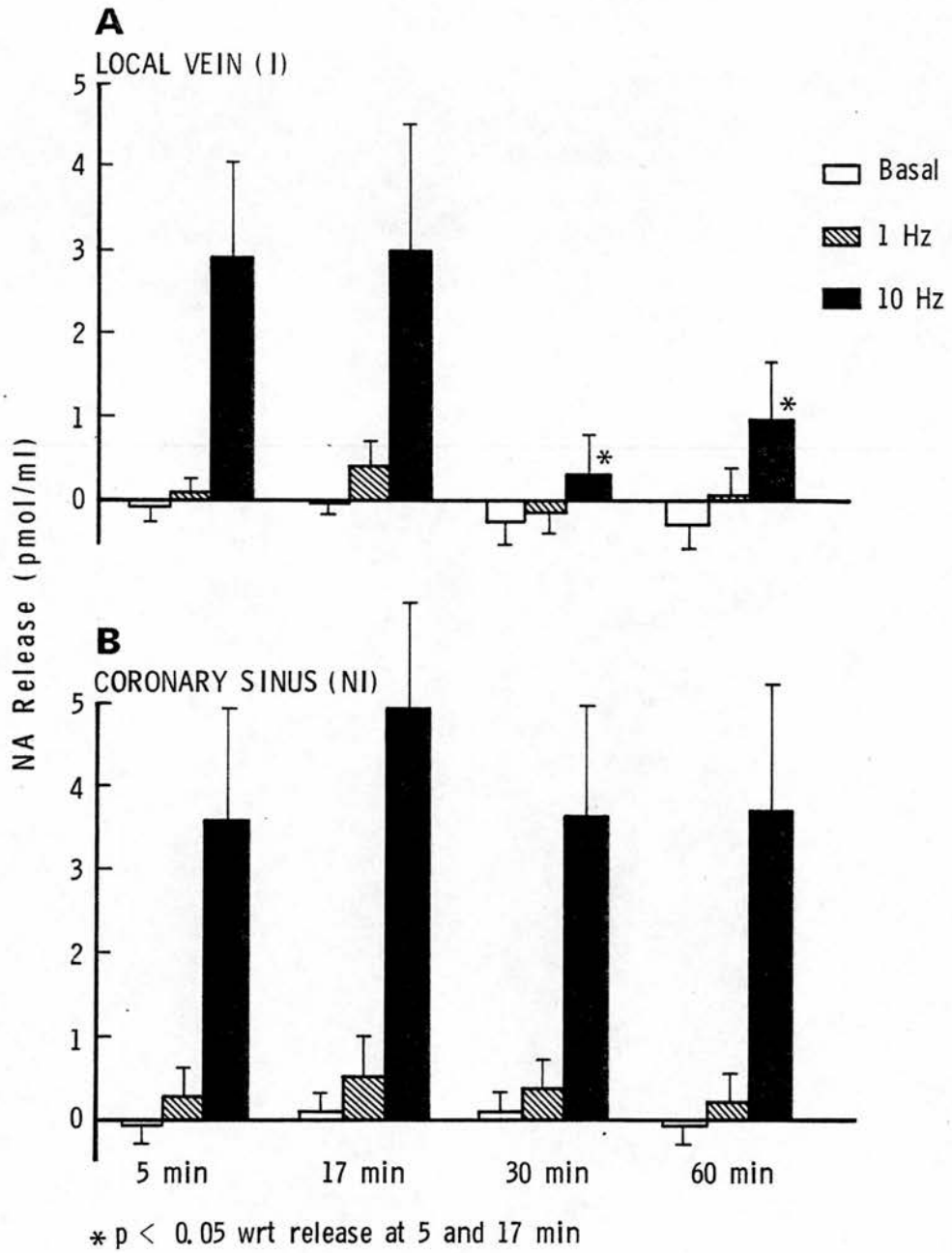


Figure 4.5 Noradrenaline release from ischaemic (A) and non-ischaemic (B) myocardium with ansa stimulation. Data from Figure 4.4.

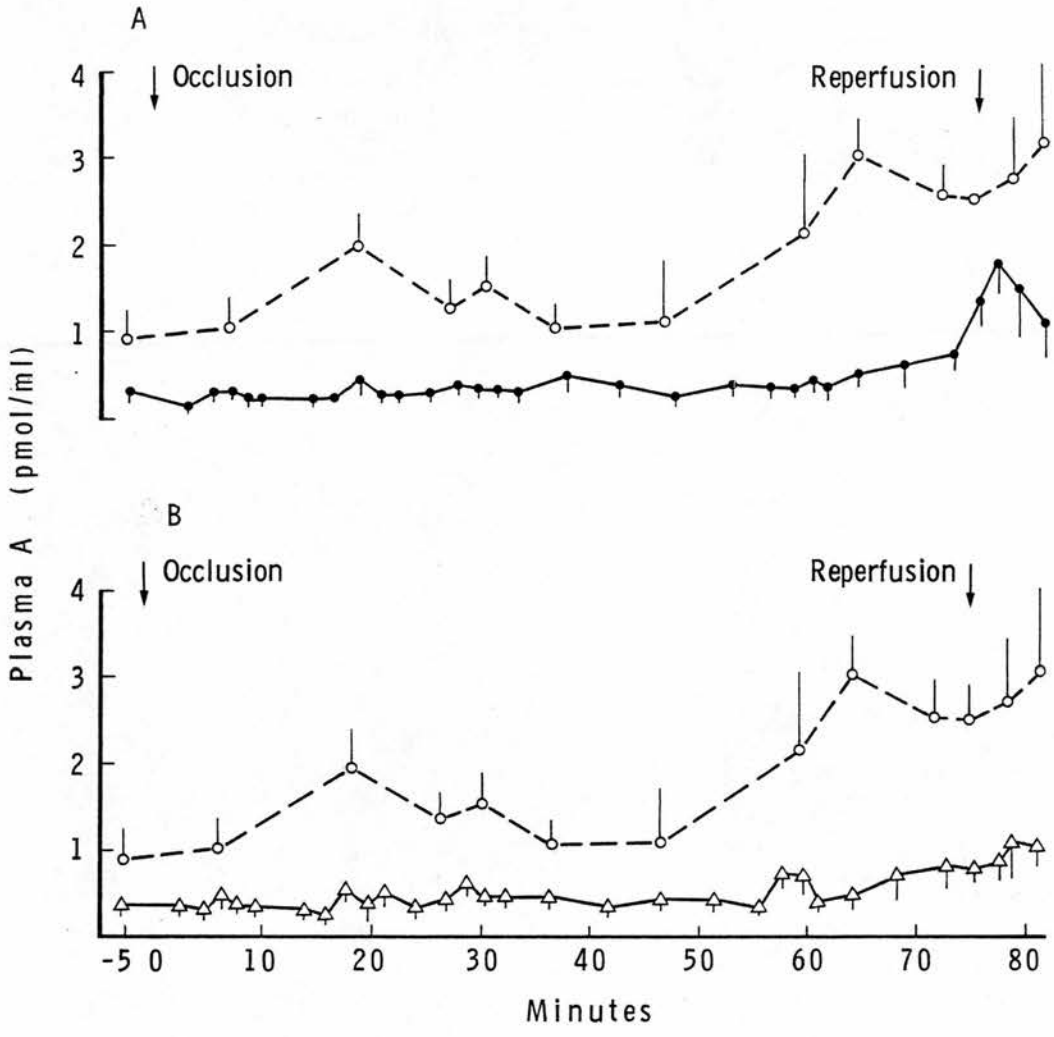


Figure 4.6 Adrenaline responses in arterial (open circles), local venous (closed circles A) and coronary sinus (triangles, B) plasma during LAD occlusion and on reperfusion (Group II, n=9).

activation delay from 44 ± 6 msecs to 56 ± 6 msecs ($p < 0.05$) and after 17 minutes activation delay also increased from 41 ± 4 to 50 ± 5 msecs ($p < 0.05$). In contrast, activation abnormalities were unchanged with ansa stimulation after 30 or 60 minutes of ischaemia, with a small but statistically non-significant reduction in activation times at these two periods. Conduction abnormalities regressed rapidly on coronary reperfusion. The change in activation time following ansa stimulation at 5, 17, 30 and 60 minutes of ischaemia is shown for each experiment in Figure 4.7. The temporal variability in this marker of electrophysiological abnormality is evident.

An index of the effect of ansa stimulation on epicardial conduction in ischaemic myocardium was derived from comparison of the product of mean activation delay across the ischaemic area and number of electrodes within the ischaemic area before and after sympathetic stimulation. As shown in Figure 4.8, this product was related to myocardial [NA] release at each early period of ansa stimulation. Release 5 and 17 minutes after coronary occlusion was associated with an increase in the epicardial activation delay index in 17 of the 18 periods of ansa stimulation. In contrast, stimulation at 30 and 60 minutes resulted in a minor increase in activation delay index on eight occasions and a decrease on ten occasions. Catecholamine release was correspondingly diminished at these times.

Lactate extraction was maintained across the non-ischaemic myocardium during LAD occlusion and reperfusion (Table 4.4). Ansa stimulation increased lactate release from ischaemic myocardium 5, 17 and 60 minutes after occlusion ($p < 0.05$ wrt prestimulation

DURATION OF ISCHAEMIA		EPICARDIAL ACTIVATION DELAY IN ISCHAEMIA AREA (msecs)	
		PRE SNS	POST SNS
5 min	$\bar{x}$	44	56
	SEM	6	6
17 min	$\bar{x}$	41	50
	SEM	4	5
30 min	$\bar{x}$	42	40
	SEM	3	3
60 min	$\bar{x}$	41	38
	SEM	4	3
74 min	$\bar{x}$	37	--
	SEM	3	
Reperfusion 1min	$\bar{x}$	32	--
	SEM	3	
Reperfusion 3min	$\bar{x}$	28	--
	SEM	2	

Table 4.3: Mean epicardial activation delay in the ischaemic area and responses to sympathetic stimulation (SNS) during coronary occlusion and on reperfusion (n = 9). The number of electrodes within the ischaemic area (see Methods for definition) varied between 5 and 56 (mean 31) for individual experiments.

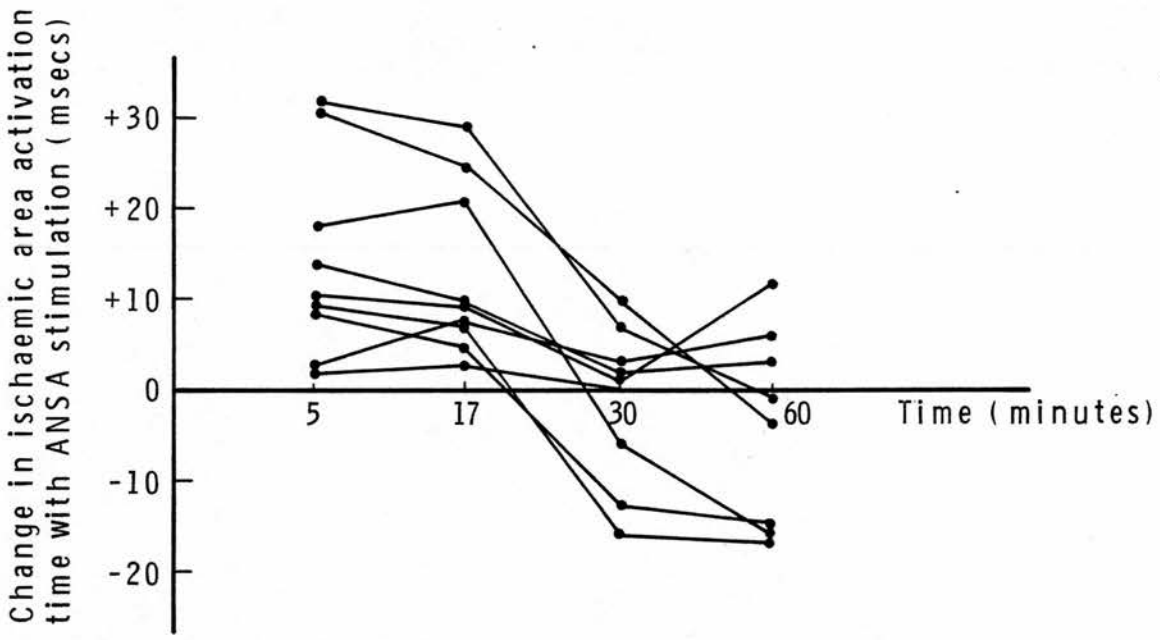


Figure 4.7 Changes in regional epicardial activation times with ansa stimulation (10 Hz for one minute) 5, 17, 30, and 60 minutes after LAD occlusion. Data from nine individual experiments.

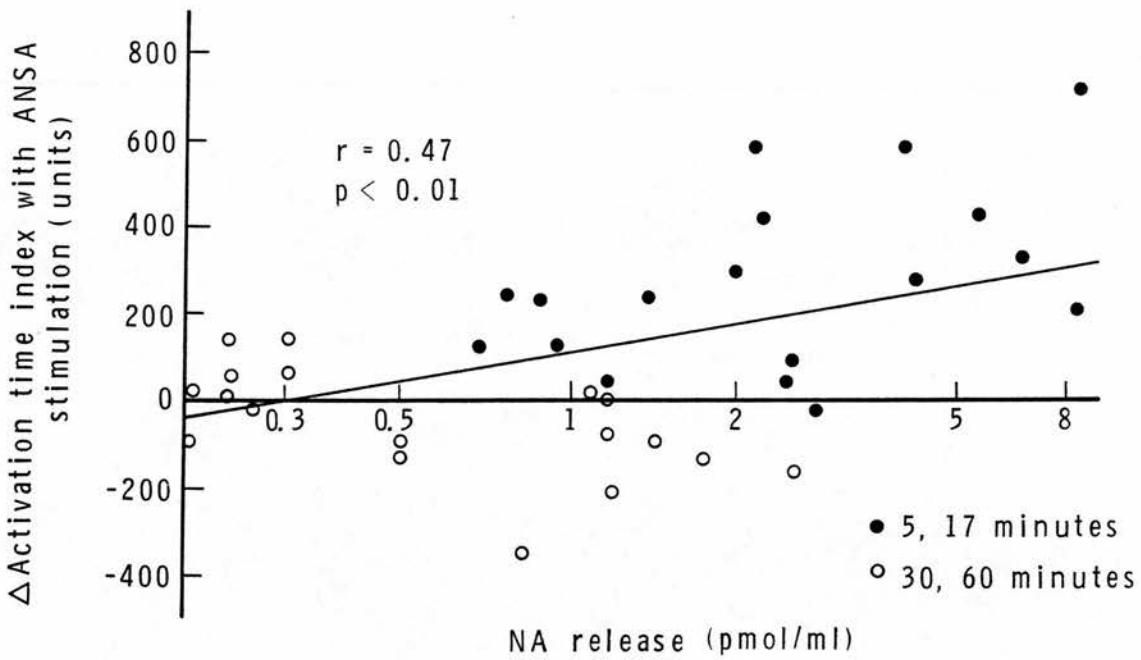


Figure 4.8 Changes in epicardial activation delay index (mean delay x no electrodes in ischaemic area) and NA overflow from the ischaemic area during ansa stimulation 5 and 17 minutes (closed circles) and 30 and 60 minutes (open circles) after coronary occlusion.

release). The increase in lactate release at 60 minutes occurred despite absence of significant [NA] release from ischaemic myocardium at this time.

Blood pressure immediately before and during ansa stimulation was not modified by coronary occlusion (Table 4.4). Mean blood flow (measured three minutes after LAD occlusion) within the ischaemic area was reduced to 18 ± 7 ml/min/100 g in endocardium and 26 ± 9 ml/min/100 g in epicardium. Flow in the non-ischaemic endocardium and epicardium was 107 ± 6 ml/min/100 g and 110 ± 11 ml/min/100 g respectively.

DISCUSSION

This study has assessed temporal variability in [NA] and [A] release from ischaemic and non-ischaemic areas of myocardium and shown that local neurotransmission is maintained over the first twenty minutes of regional ischaemia but selectively inhibited in the ischaemic region thereafter. Furthermore, evidence for a detrimental effect of sympathetic stimulation on local epicardial activation abnormalities within the ischaemic area is demonstrable only during nerve stimulation five and seventeen minutes after LAD ligation, the times of maximum likelihood of spontaneous arrhythmias in this model (Kaplinsky et al, 1979). At these two early periods of enhanced vulnerability, a crude relationship is evident between the extent of local [NA] release during sympathetic stimulation, and the resulting increase in local activation times. Assuming that flow within the ischaemic region remains relatively constant with time (at least over 75 minutes), the reduction in peak [NA] levels in ischaemic venous effluent following sympathetic

DURATION OF ISCHAEMIA		MYOCARDIAL LACTATE EXTRACTION (%) I	NI	BLOOD PRESSURE (mmHg)
Basal	$\bar{x}$	+31	+41	122
	SEM	10	12	16
5 min	$\bar{x}$	-93	+46	120
	SEM	29	11	14
SNS	$\bar{x}$	-246	+19	
	SEM	40	17	
17 min	$\bar{x}$	-153	+18	116
	SEM	31	13	9
SNS	$\bar{x}$	-229	+14	142
	SEM	30	12	17
30 min	$\bar{x}$	-127	+19	113
	SEM	37	9	13
SNS	$\bar{x}$	-197	+15	139
	SEM	42	12	12
60 min	$\bar{x}$	-142	+32	109
	SEM	26	14	9
SNS	$\bar{x}$	-200	+30	138
	SEM	37	16	12
74 min	$\bar{x}$	-125	+27	106
	SEM	19	18	10
Reperfusion	$\bar{x}$	-75	+6	107
	SEM	29	10	13

Table 4.4: Myocardial lactate extraction $\left(\frac{\text{art} - \text{ven}}{\text{art}} \times 100\right)$ and blood presssure responses before and during LAD occlusion, sympathetic stimulation (SNS) and on reperfusion (Group II; n = 11).

stimulation after 30 minutes of occlusion represents an eighty per cent inhibition of [NA] release from the heart in comparison to responses at five and seventeen minutes. Basal [NA] levels do not change with coronary occlusion beyond thirty minutes suggesting that extraction is unaltered at this time. Enhanced extraction of [A] across ischaemic myocardium is maintained throughout the longer occlusion. It is likely that the contribution to ischaemic effleunt from collaterals that have not perfused ischaemic tissue is small as the dissociation between catecholamine release from anterior (ischaemic) and lateral (non-ischaemic) surfaces of the heart was readily apparent after thirty minutes. Furthermore, lactate release was confined to ischaemic effluent.

Although continuous stimulation of cardiac sympathetic nerves for periods of six to ten minutes has been reported to cause depletion of myocardial catecholamines (Pindok et al, 1981) and reduction in peak myocardial [NA] overflow (Levy and Blattberg, 1976) sequential one minute periods of stimulation result in reproducible, frequency dependent [NA] overflow from both anterior and lateral surfaces of the heart without evidence of tachyphylaxis. Thus, despite large interindividual differences in peak [NA] overflow, this technique provides a reliable means of assessing the functional responses of the neurosympathetic axis in both ischaemic and non-ischaemic areas of myocardium. No significant reduction in local venous [NA] release was observed following dissection of the coronary artery, showing that this manoeuvre does not result in regional cardiac denervation, one theoretical explanation for absence of spontaneous neurotransmitter release during ischaemia. The model also provides a means for testing drug and metabolite influences on the nerve terminal

(Yamaguchi et al, 1977; Chapters 5 and 6).

Although the mechanism of regional impairment of neurotransmission after thirty minutes coronary occlusion has not been elucidated, it is unlikely to have been secondary to depletion of neurotransmitter from the nerve terminal as [NA] levels decline following coronary occlusion at a rate between three and ten per cent per hour (Mathes and Gudbjarnason, 1971; Serrano et al, 1974), too slow to influence responses by thirty minutes. Histochemical evidence of peripheral sympathetic denervation is not detectable until between two and six hours of ischaemia (Terravainen and Makitie, 1976; Muntz et al, 1981). Furthermore, massive release of neurotransmitter during reperfusion (Figure 4.4) argues against depletion of nerve terminal stores by ischaemia of this duration. Lactate release, by contrast, decreased rapidly during reperfusion suggesting that neurotransmitter release at this time was from preformed stores and not merely a part of the reperfusion-induced washout of ischaemic metabolites. It is most likely that accumulation of one or several metabolites inhibits neurotransmission either at the level of the nerve terminal or more proximally as nerve fibres pass through ischaemic tissue. Vagal tone may potentially inhibit [NA] release through a peripheral muscarinic mechanism (Levy and Blattberg, 1976, 1979; Lavallee et al, 1980) but has little effect on the spontaneous release of [NA] and [A] from ischaemic myocardium (Williford et al, 1983). Vagal tone, however, is low during barbiturate anaesthesia (Priano et al, 1969) is preferentially enhanced by stimulation of mechanoreceptors along the inferior, rather than the anterior surface of the heart (Thoren, 1979) and is unlikely to be selectively active in inhibiting catecholamine release specifically from the ischaemic

area after thirty minutes.

The findings of this study agree well with data from Martins et al (1980) who showed dissociation between the effects of left stellate ganglion stimulation and systemic noradrenaline infusion on regional myocardial shortening thirty minutes after coronary occlusion. Systolic expansion of the ischaemic territory of the left ventricle was decreased during the catecholamine infusion (at a dose producing a similar increase in blood pressure to sympathetic stimulation) while systolic expansion was unaltered by stellate stimulation. Responses before thirty minutes of ischaemia and catecholamine release from the heart were not assessed. Although these two techniques of cardiac activation are not directly comparable, the results suggested impaired neurotransmission within the ischaemic region at this time. Similar abnormalities in sympathetic nerve function have been shown in a preliminary report by Cuiffo et al (1983) after twenty-five minutes of coronary occlusion and reperfusion.

Coronary reperfusion after occlusion for ten or seventy-five minutes is associated with substantial neurotransmitter release from the ischaemic area. As the regional venous sampling technique cannot completely separate ischaemic and non-ischaemic effluent, particularly during the reactive hyperaemia associated with coronary reperfusion, it is not possible to determine with certainty the origin of the small reperfusion-induced release of [NA] into the lateral (non-ischaemic) vein after the longer occlusion. It is likely, however, that a significant portion of this overflow represents spillover from the ischaemic area. Theoretically, [NA] release on reperfusion may result from washout

of neurotransmitter released from an extraneuronal compartment during the period of ischaemia or from release directly associated with reperfusion. The latter process is likely to predominate because of the similar time course of rise and fall in levels of [NA] in ischaemic and non-ischaemic effluent following sympathetic stimulation. If released neurotransmitter did not reach the vascular bed in the ischaemic area, then the time course for [NA] release from this area should have been longer and the maximum concentrations lower than in the absence of ischaemia or in conparison to the response in the non-ischaemic area. Extraction of [A] decreased during reperfusion, compatable with either a reduction in myocardial [A] uptake, possibly secondary to hyperaemia, or coexisting release of [A] at this time.

It is probable that the fundamental electrophysiological mechanisms of the two early phases of arrhythmogenesis are different. It is now generally accepted that the earlier phase 1a arrhythmias are reentrant in origin, although circus movements may under certain circumstances be generated by regional flow of injury current between ischaemic and non-ischaemic areas (Janse et al, 1980). Several studies have demonstrated fractionation and fragmentation of surface and intramural activation (Boineau and Cox, 1973; Scherlag et al, 1974; Russell et al, 1979; Janse and Kleber, 1981) and other phenomena associated with reentry. Spatial heterogeneity of activation may be a further marker for arrhythmogenesis at this time (Russell et al, 1983). In contrast, diastolic bridging activity associated with reentry is absent during phase 1b arrhythmias and responses to pharmacological interventions may be different. Phase 1b arrhythmias are, apparently, more effectively suppressed by calcium antagonists

(Parratt, 1982) and less effectively suppressed by beta-adrenoceptor blockade (Menken et al, 1979) than phase 1a arrhythmias. Epicardial activation delay has been shown to be less during phase 1b than phase 1a arrhythmias in this and in a previous study (Kaplinsky et al, 1979). However, the present study has demonstrated that neurosympathetic responsiveness is maintained during both early phases of arrhythmias. Clearly, the electrophysiological consequences of catecholamine release in ischaemic myocardium are critically dependent on the duration of ischaemia. Metabolic and ionic shifts such as potassium concentration, pH, redox state, glycolytic activity and accumulation of toxic metabolites such as lysophosphatides or fatty acyl carnitine can all contribute to depression of conduction in ischaemic myocardium and may be modified by catecholamine release.

Temporal variability in neurosympathetic activity across the myocardium may have important implications in the treatment and prevention of arrhythmias following acute ischaemia in man. If similar phases of early arrhythmias operate, and if adrenoceptor modulation in ischaemic tissue is a prerequisite for an antiarrhythmic action of a sympatholytic drug (suppositions that remain to be proved), then efficacy will depend on delivery to ischaemic tissue before the development of impaired neurotransmission and low risk of lethal arrhythmias. Conversely, if such drugs act primarily on non-ischaemic myocardium, then efficacy will be independent of the duration of ischaemia. In view of the marked time-dependence of the appearance of spontaneous arrhythmias in the dog and many other animal models, it is probable that ischaemic tissue is the primary target for drug action.

This study has provided a link between catecholamine release from the heart and the electrophysiological responses of ischaemic tissue to sympathetic stimulation and highlighted temporal variability in neurosynaptic activation during the first hour of ischaemia. Further studies will be necessary to clarify the metabolic determinants of impaired neurotransmisson after thirty minutes and the causes and pathophysiological consequences of myocardial catecholamine release associated with coronary reperfusion.

5 PRESYNAPTIC CONTROL OF CATECHOLAMINE RELEASE: INFLUENCE OF REUPTAKE AND ALPHA ADRENOCEPTOR BLOCKADE

The importance of both active and passive mechanisms in the disposition of [NA] released from adrenergic nerve terminals has been recognised for many years. The neuronal cell membrane possesses a powerful amine reuptake pump, capable under basal conditions of the removal of approximately three quarters of arterial [NA] in a single circulation through the heart (Chidsey et al, 1963), and therefore in close apposition to the vascular compartment. Following reuptake, the neurotransmitter may be conserved within storage vesicles or deaminated by intramitochondrial monoamine oxidase, both processes requiring ATP. Alternatively, myocytes and vascular smooth muscle cells actively take up [NA] which, once in the cytoplasm, is inactivated mainly through O-methylation to normetanephine (Chidsey et al, 1963; Kalsner 1975, 1976; Verbeuren et al, 1977).

The importance of reuptake and enzymatic degradation in the removal of released neurotransmitter is undoubtedly modified both by changes in sympathetic activation and changes in passive removal mechanisms. Tyramine for example, increases four fold the release of [NA] from the isolated canine heart while increases in normetanephrine release are minor (Chidsey et al, 1963), suggesting that the capacity of enzymatic processes to inactivate catecholamines may be limited. It is also likely that blood flow per se is a determinant of neurotransmitter removal by reuptake, enzymatic degradation, or passive diffusion into the vascular compartment. The studies in Chapter 3, for example, showed enhanced extraction of [A] across ischaemic myocardium during coronary occlusion, and reduced extraction on reperfusion, compatible with [A] removal being dependent on circulation time.

More recently, evidence has become available of the presence of adrenoceptors on the outer surface of the adrenergic nerve terminal membrane. These adrenoceptors are capable of acting as powerful local modulators of catecholamine release from nerve terminals. In 1957, Brown and Gillespie first reported (although they did not appreciate the significance of their report) that phenoxybenzamine, an alpha-adrenoceptor blocking drug, increased the stimulation induced overflow of [NA] in the perfused cat spleen. In 1971, four independent reports from three continents were published each suggesting that [NA] release was locally regulated by a negative feedback mechanism (Farnebo and Hamberger, 1971; Kirpekar and Puig, 1971; Langer et al, 1971; Starke, 1971). Two types of experimental evidence supported this contention; firstly, augmentation of release by alpha-adrenoceptor antagonists in excess of that observed by preventing uptake into nerves or muscle cells, and secondly, diminished release of neurotransmitter by the administration of alpha-adrenoceptor agonists. Rank order potencies of alpha-adrenoceptor agonists in inhibiting adrenergic neurotransmitter release (presynaptic action) or constricting vascular smooth muscle (postsynaptic action) have shown ratios up to 300, methoxamine and phenylephrine favouring the postsynaptic (alpha 1) receptor whereas clonidine, oxymetazoline and tramazoline are more active at the presynaptic (alpha 2) site. More recently, postsynaptic alpha adrenoceptors with characteristics of the alpha 2 subtype have also been recognised in certain tissues (Drew and Whiting, 1979; Starke and Langer, 1979), although their physiological significance is less certain.

The release of [NA] from cardiac tissues may also be changed by drugs with affinity for the beta-adrenoceptor. In general,

agonists increase whereas antagonists reduce nerve-stimulation induced neurotransmitter release (Adler-Graschinsky and Langer, 1975), although the latter action in particular is controversial (Majewski et al, 1980, 1981). The role of presynaptic beta-adrenoceptors in modulating [NA] release from ischaemic myocardium is not considered further in this chapter.

Absence of spontaneous catecholamine overflow from the heart (Chapter 3) despite apparently normal neurosympathetic activity at the time of early ventricular arrhythmias implies (Chapter 4) an important role for local modulators of catecholamine release in acutely ischaemic myocardium, the modulators acting to limit neurotransmitter overflow into ischaemic effluent. The studies presented in this chapter have therefore investigated the influences of an alpha 2 adrenoceptor antagonist (yohimbine) and two reuptake inhibitors (viloxazine and desmethylimipramine), on spontaneous and nerve stimulated catecholamine overflow and on arrhythmias during acute ischaemia.

Methods, Protocols

Adult mongrel dogs of either sex were anaesthetized with pentobarbitone (25 mg/kg intravenously for induction; 4 mg/kg/hr for maintenance) and prepared surgically as described in Chapter 4 with the addition of a second left atrial cannula for those experiments requiring labelling of the heart with 1-^{3}H-noradrenaline. Myocardial catecholamine and lactate extraction and regional overflow were measured before and during ten or twelve minute periods of LAD ligation and on reperfusion before and in the presence of the reuptake inhibitors and alpha 2

adrenoceptor antagonist both singly and in combination. In addition, graded stimulation of the left stellate ganglion was performed before and during coronary occlusion in the experiments investigating yohimbine and desmethylimipramine, and regional epicardial activation times were measured before and immediately after each period of high frequency ansa stimulation.

Group I: Yohimbine

A test occlusion was performed initially to determine the area of ischaemia and patterns of electrophysiological abnormality. Minute-to-minute changes in plasma catecholamines across ischaemic and non-ischaemic areas of myocardium were measured in nine experiments during a twelve minute period of coronary occlusion and for two minutes of reperfusion (Figure 5.1). The anterior and posterior ansa from the left stellate ganglion were stimulated for one minute (as described previously) at low (1 Hz) and at high (10 Hz) frequency four and six minutes respectively following coronary occlusion. Blood samples (up to 1 ml) for catecholamine analysis were withdrawn simultaneously from arterial and venous sampling sites over 15-30 seconds at the times shown in Figure 5.1a. Full analysis was possible with 200 µl. Regional myocardial blood flow was measured three minutes after occlusion and conduction delay was measured prior to coronary occlusion, at three minutes after occlusion and immediately after low and high frequency ansa stimulation. Ventricular fibrillation was managed by internal DC defibrillation (10 joules) after removal of the coronary occlusion clip. All samples obtained after spontaneous venticular fibrillation were excluded from the analysis.

One hour after coronary reperfusion, the protocol was repeated

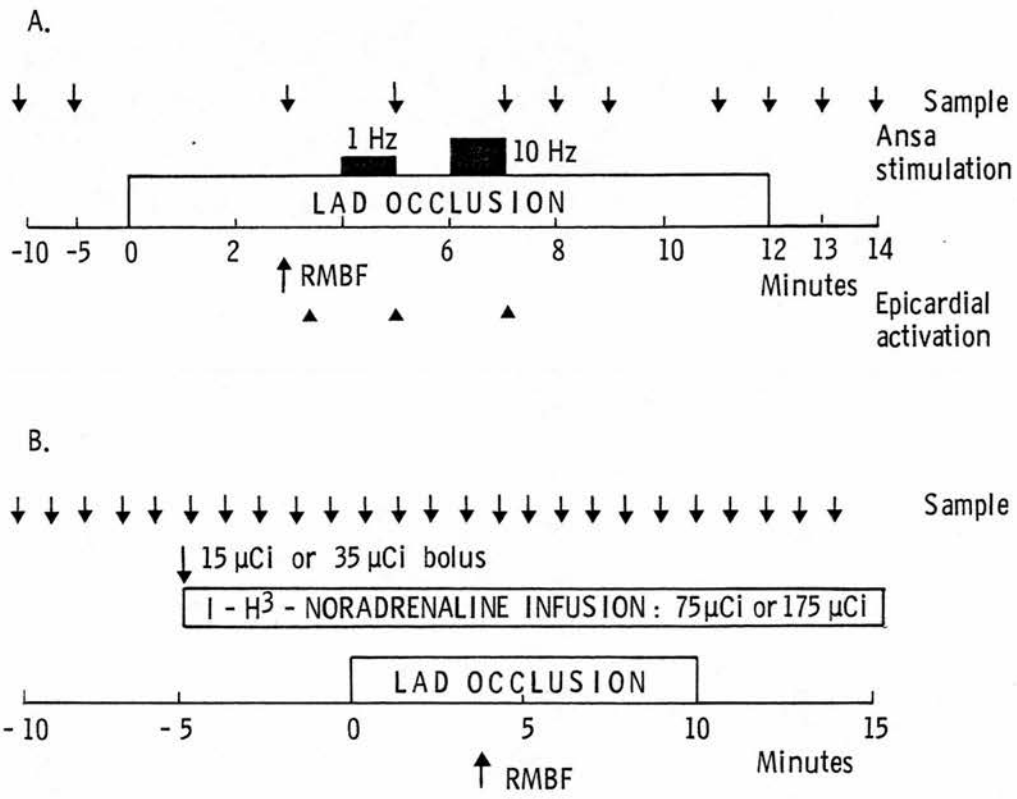


Figure 5.1 Experimental protocols. In Groups I, II and III (A), regional catecholamine overflow and electrophysiological responses were evaluated during a twelve minute LAD occlusion and on reperfusion before and after administration of desmethylimipramine and yohimbine. In Group IV (B), regional noradrenaline extraction was measured during a ten minute LAD occlusion and on reperfusion before and after viloxazine by prelabelling the heart with I-H^3-noradrenaline, 75µCi for the first (control) and 175µCi for the second (viloxazine) occlusion.

in the presence of yohimbine (1 mg/kg i.v. bolus; 0.2 mg/kg/hr infusion) as the hydrochloride salt, commenced 30 minutes prior to the second occlusion. Previous studies from our laboratory have shown that patterns of electrophysiological abnormality are reproducible for the second and subsequent occlusions (Russell et al, 1979), thus allowing comparison of the effect of drug interventions in the same experimental preparation. The dose of yohimbine was selected following evaluation of responses at three doses in the absence of coronary occlusion (Table 5.1). [NA] release at 1 Hz stimulation increased after low-dose yohimbine (0.25 mg/kg), but no further increments were apparent with increasing yohimbine dose. At 10 Hz stimulation, overflow was further potentiated by the 1.0 mg/kg dose but not by the 3.0 mg/kg dose. As a result of these preliminary observations, 1.0 mg/kg yohimbine was selected for study during myocardial ischaemia.

Group II: Desmethylimipramine

The same protocol as above was used for six experiments before and after intravenous desmethylimipramine 2.5 mg/kg bolus and 1 mg/kg/hr maintenance infusion.

Group III: Yohimbine/desmethylimipramine

The effects of both drugs in combination at the dosages detailed above were evaluated in seven experiments during a third twelve minute period of ischaemia with a similar protocol of sampling and sympathetic stimulation. Regional blood flow measurements were not undertaken in this group. The same experimental preparation as Group I was used for these studies although in two experiments a third period of ischaemia was not

YOHIMBINE DOSE (mg/kg i.v.)	MYOCARDIAL NA RELEASE (negative A-V difference; pmol/l; n = 3)			
	Basal	1 Hz	Basal	10 Hz
Control	0.58±0.23	1.33±0.35	0.23±0.43	14.14±8.91
0.25	-0.11±0.36	+4.01±1.08	0.27±0.43	+24.07±8.86
1.0	-0.65±0.38	+3.04±1.54	0.44±0.89	+30.30±10.32
3.0	0.45±0.78	+2.87±1.43	0.69±0.70	+27.23±14.53

+ $P < 0.01$ compared to control

Table 5.1: Effects of yohimbine on myocardial NA release (mean ± SEM) without ischaemia. The left stellate ganglion was stimulated at low (1 Hz) and high (10 Hz) frequency for one minute. The mean NA concentration was determined in arterial and local venous plasma before and over two minutes after ansa stimulation.

undertaken because of haemodynamic deterioration in the preparation associated with excessive blood loss and a low haematocrit.

Group IV

For this series, the influence of the specific neuronal reuptake blocking agent viloxazine (ICI Pharmaceuticals, England) on regional myocardial ^{3}H-noradrenaline extraction was evaluated in twelve experiments. Following basal sampling over five minutes, myocardial labelling was achieved by a left atrial bolus injection of 15 μCi 1-H^3-noradrenaline (Amersham International, England; Ref. TRK584, Spec Act 30-50 Ci/mmol) dissolved in fresh 1% ascorbic acid followed by a continuous atrial infusion of 60 μCi over twenty minutes (Figure 5.1B). The total dose infused was approx. 2 nmol per dog. The infusion was designed to keep arterial H^3-noradrenaline levels constant. No attempt was made to separate radioactive metabolites of noradrenaline from the parent amine. A ten minute period of LAD occlusion was performed five minutes after the bolus injection with minute-to-minute regional sampling throughout the occlusion and for five minutes on reperfusion. Regional myocardial blood flow was determined four minutes after occlusion. After a thirty minute recovery period, intravenous viloxazine at two doses [5 mg/kg (n = 6) or 25 mg/kg (n = 3) bolus and 1 mg/kg/hr or 5 mg/kg/hr maintenance infusion respectively] was given. In a second group of controls (n = 3), isotonic saline was infused. Thirty minutes after drug or saline administration, a second period of continuous sampling was commenced over five minutes to quantify residual radioactivity. A second intraatrial bolus injection of 35 μCi 1-H^3 noradrenaline was given followed by a continuous infusion of 140 μCi over twenty minutes. The higher

dose of radioactivity (which had no demonstrable haemodynamic effect) was required to minimise the influence of residual radioactivity on measurement of myocardial noradrenaline extraction during the second occlusion. Coronary occlusion, reperfusion and regional blood flow estimation were performed as for the first period of ischaemia.

Changes in catecholamine and lactate concentrations following drug administration were evaluated by analysis of variance and computed modified t-statistic for pair differences. Non parametric methods (Wilcoxon test for pair differences) were used for analysis of changes in blood flow and surface electrophysiology. Data are expressed as mean $\pm$ standard error of the mean with a five per cent level of confidence considered statistically significant.

RESULTS

Group I - Yohimbine

Catecholamines:

Mean [NA] concentrations at arterial and at ischaemic and non-ischaemic venous sampling sites during coronary occlusion and reperfusion are shown in Figure 5.2. Under control conditions, [NA] levels were unchanged at all sampling sites during coronary occlusion but a small reperfusion-induced rise in [NA] was observed from the previously ischaemic area as noted previously. Basal levels of [NA] were slightly increased after yohimbine, but the rise during left ansa stimulation was more marked, particularly in the ischaemic effluent, where the peak [NA] level at 10 Hz stimulation was increased from 4.3 $\pm$ 0.4 to 11.8 $\pm$ 5.4 pmol/ml (p

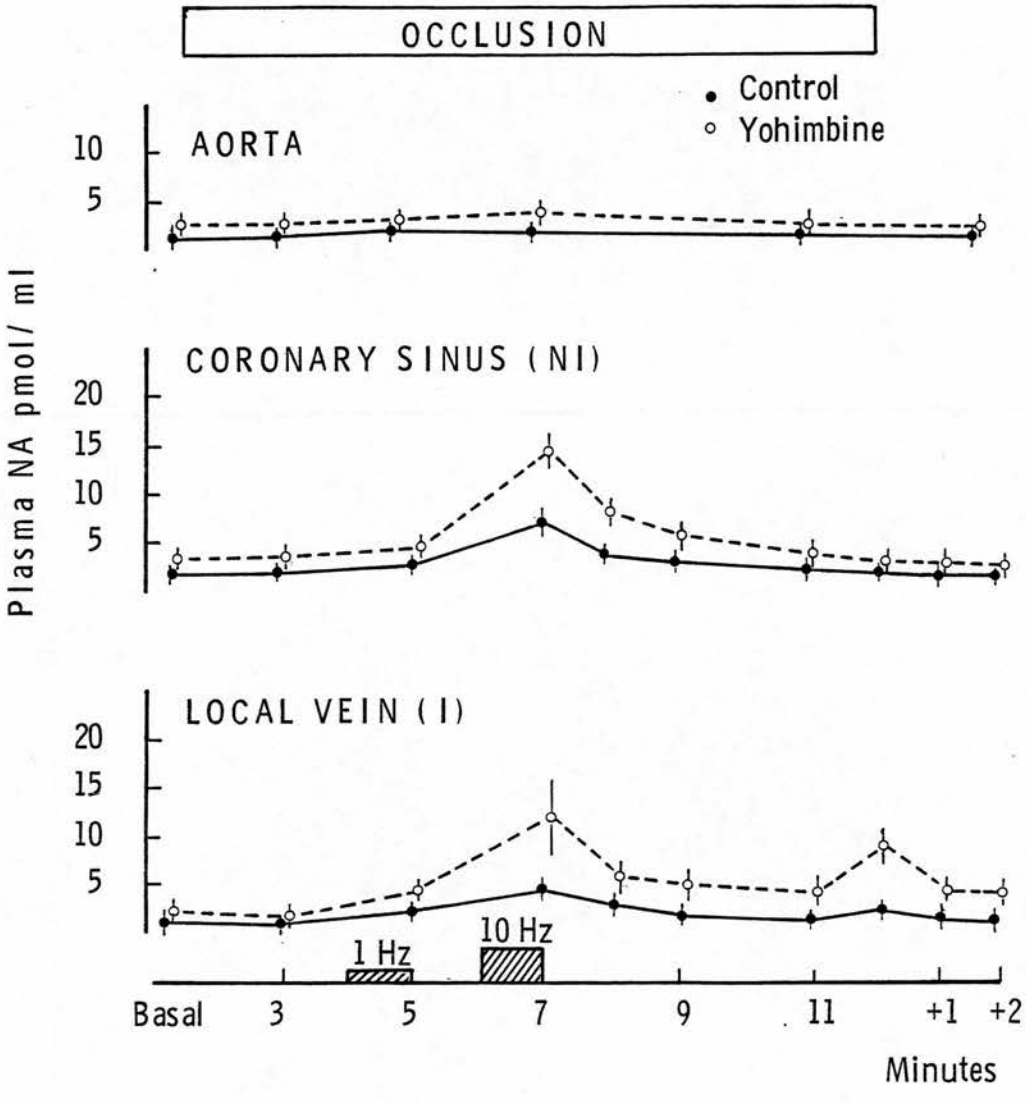


Figure 5.2 Plasma noradrenaline (NA) concentration in arterial and in non-ischaemic and ischaemic venous effluent during coronary occlusion and reperfusion (mean ± SEM; N=9); effects of yohimbine. The blocks indicate low and high frequency ansa stimulation.

< 0.005). The increase in [NA] in ischaemic effluent associated with reperfusion was similarly enhanced. Expressed as the negative arteriovenous difference, [NA] release from ischaemic myocardium was not detectable three or eleven minutes after coronary occlusion (Figure 5.3) but was significantly potentiated both with sympathetic stimulation and on reperfusion.

Extraction of [A] was maintained across both ischaemic and non-ischaemic myocardium and was not influenced by coronary occlusion or ansa stimulation (Figure 5.4). Coronary reperfusion resulted in a significant rise in [A] in ischaemic effluent (from 0.5 ± 0.2 pmol/ml one minute before to 1.1 ± 0.4 pmol/ml one minute after reperfusion ($p < 0.05$)). A smaller rise in [A] from non-ischaemic effluent during reperfusion was not statistically significant. Following yohimbine, arterial [A] rose 2-3 fold and extraction across the heart increased from 45 to 64 per cent. Enhanced extraction of [A] was maintained at both sampling sites during coronary occlusion with a rise in venous [A] during coronary reperfusion, compatible with release of [A] from the heart at this time.

Lactate:

Myocardial lactate production across ischaemic myocardium occurred throughout coronary occlusion and was increased ($p < 0.05$) during sympathetic stimulation (Figure 5.5). Net extraction of lactate was maintained across non-ischaemic myocardium, although extraction tended to fall during coronary occlusion, suggesting either a minor contribution from ischaemic effluent at this sampling site or some lactate production from the non-ischaemic

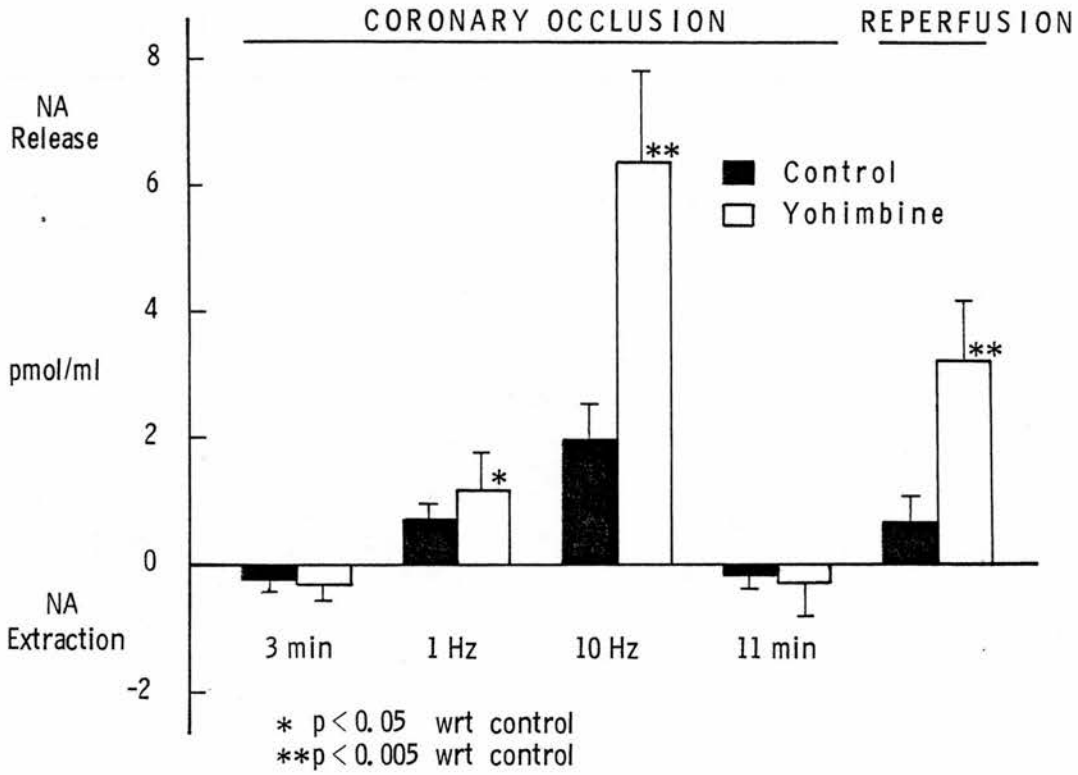


Figure 5.3 Myocardial noradrenaline (NA) release from and extraction across ischaemic myocardium during coronary occlusion and reperfusion before, during and after ansa stimulation. Data shown before and after yohimbine administration (taken from figure 5.2).

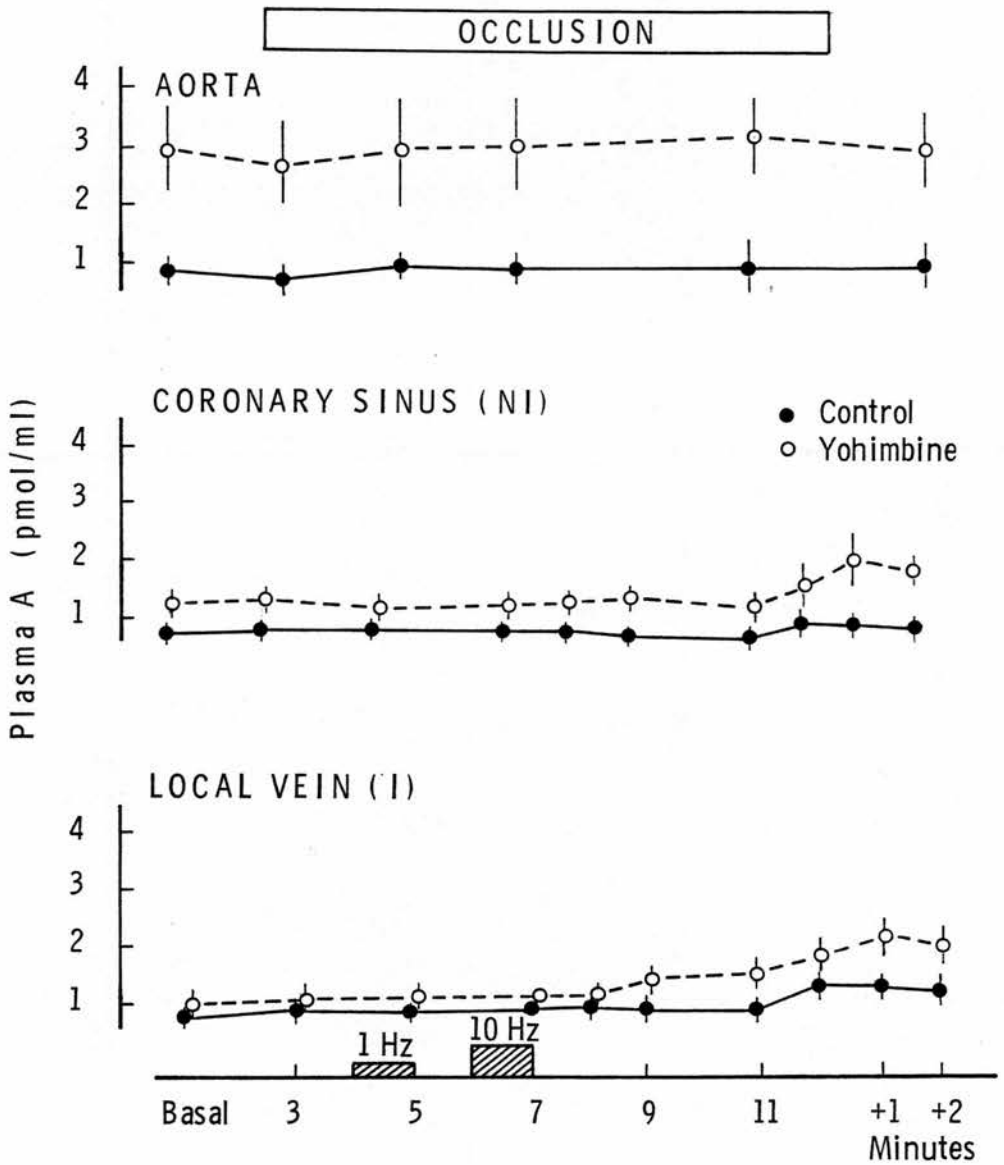
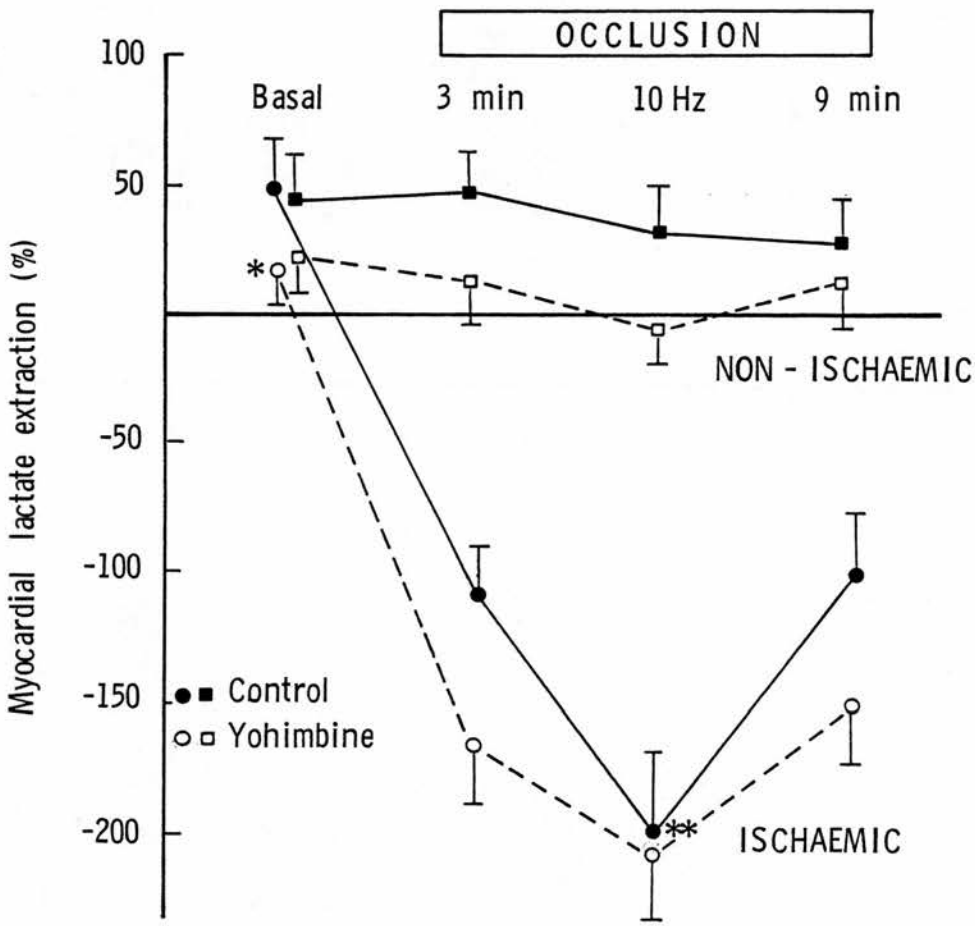


Figure 5.4 Plasma adrenaline (A) concentrations during coronary occlusion and reperfusion (mean ± SEM, n=9). Effects of yohimbine.



* $p < 0.05$ wrt control
** $p < 0.05$ wrt extraction at 3, 9 mins.

Figure 5.5 The effects of yohimbine on myocardial lactate extraction (mean ± SEM; n=9). Data shown for samples withdrawn before occlusion, three and nine minutes after occlusion and immediately after high frequency ansa stimulation.

$$\text{(extraction (\%)} = \frac{\text{Art-Ven}}{\text{Art}} \times 100\text{)}$$

area. Yohimbine reduced myocardial lactate extraction before coronary occlusion ($p < 0.05$) but did not modify the pattern of lactate release from the heart during coronary occlusion and sympathetic stimulation.

Haemodynamics:

Blood flow to non-ischaemic myocardium was not significantly affected by yohimbine. Endocardial flow fell by 9 per cent from 90 $\pm$ 14 to 82 $\pm$ 14 ml/min/100 g, while in the epicardium flow was reduced by 6 per cent from 89 $\pm$ 11 to 83 $\pm$ 15 ml/min/100 g. Neither change was statistically significant. In contrast, ischaemic endocardial flow decreased by 24 per cent from 27 $\pm$ 2 to 20 $\pm$ 11 ml/min/100 g ($p < 0.01$) and ischaemic epicardial flow decreased 36 per cent from 43 $\pm$ 17 to 28 $\pm$ 14 ml/min/100 g ($p < 0.01$). In keeping with some post synaptic alpha-adrenoceptor blockade, mean arterial blood pressure fell from 108 $\pm$ 8 to 84 $\pm$ 7 mmHg ($p < 0.01$) after yohimbine. The increment in mean blood pressure with ansa stimulation at both low (4 $\pm$ 4 mmHg control; 9 $\pm$ 7 mmHg yohimbine) and high (14 $\pm$ 16 mmHg control; 19 $\pm$ 8 mmHg yohimbine) frequency stimulation was not significantly affected.

Electrophysiology:

Spontaneous ventricular fibrillation occurred twice during control occlusions (22 per cent) and five times after yohimbine (56 per cent). In keeping with this tendency for an increased incidence of spontaneous ventricular fibrillation the mean duration of coronary occlusion without ventricular fibrillation decreased

after yohimbine from 10 ± 1 to 8 ± 1 minutes ($p < 0.05$).

Regional epicardial activation abnormalities after coronary occlusion were intensified by yohimbine. For each experiment, the mean conduction delay was compared at electrodes within the central ischaemic zone. Three minutes after coronary occlusion, yohimbine increased activation delay by 26 per cent from 41 ± 2 to 54 ± 5 ms ($p < 0.01$). At low and high frequency ansa stimulation, activation delay increased by 24 and 22 per cent respectively (from 51 ± 3 ms before yohimbine to 67 ± 7 ms after yohimbine at 1 Hz, and from 57 ± 4 ms before yohimbine to 73 ± 10 ms after yohimbine at 10 Hz [$p < 0.01$]). The ischaemic zone activation delays are summarised for each experiment in Table 5.2.

Patterns of activation abnormality from one experiment are illustrated in Figure 5.6. Prior to coronary occlusion most local activation times were less than 30 ms. After three minutes of ischaemia, both the area and magnitude of the conduction abnormalities that developed across the ischaemic area were increased by yohimbine (Figure 5.6a). Sympathetic nerve stimulation induced a further increase in local activation times during the control occlusion but no site showed delay in excess of 60 ms. After yohimbine, however, approximately 10 per cent of sites showed delay greater than 60 ms. Profiles of conduction changes across the ischaemic zone from the same experiment (Figure 5.6b) showed an increase in the magnitude and variability in activation delay in the ischaemic zone. These changes in activation times are in keeping with an increase in spontaneous arrhythmias. In the example shown, spontaneous ventricular fibrillation occurred two minutes after high frequency ansa

EXPT	ELECTRODES	EPICARDIAL ACTIVATION DELAY					
		CONTROL			YOHIMBINE		
		3 min	1Hz	10Hz	3 min	1Hz	10Hz
(1)	18	53 ± 6	66 ± 8	79 ± 8	89 ± 8	107 ± 6	117 ± 3
(2)	15	39 ± 2	43 ± 2	42 ± 2	54 ± 4	56 ± 4	65 ± 6
(3)	13	38 ± 4	47 ± 5	49 ± 2	50 ± 4	59 ± 4	71 ± 4
(4)	15	43 ± 8	51 ± 5	59 ± 8	57 ± 5	80 ± 5	83 ± 5
(5)	23	33 ± 1	48 ± 3	63 ± 3	38 ± 1	59 ± 4	VF
(6)	16	34 ± 2	38 ± 3	41 ± 3	39 ± 3	47 ± 3	56 ± 3
(7)	20	49 ± 5	67 ± 5	VF	59 ± 6	VF	-
(8)	15	36 ± 3	44 ± 3	60 ± 3	45 ± 3	50 ± 5	49 ± 4
(9)	17	41 ± 6	53 ± 4	60 ± 8	60 ± 7	77 ± 7	VF
Mean ± SEM		41 ± 2	51 ± 3	57 ± 7	54 ± 5	67 ± 4	73 ± 10

Table 5.2: Mean epicardial activation delay before and after sympathetic stimulation in the central ischaemic area. The number of electrode sites analysed within the ischaemic area for each experiment is shown in the left hand column. In three studies, conduction maps were not completed with nerve stimulation because of ventricular fibrillation (VF).

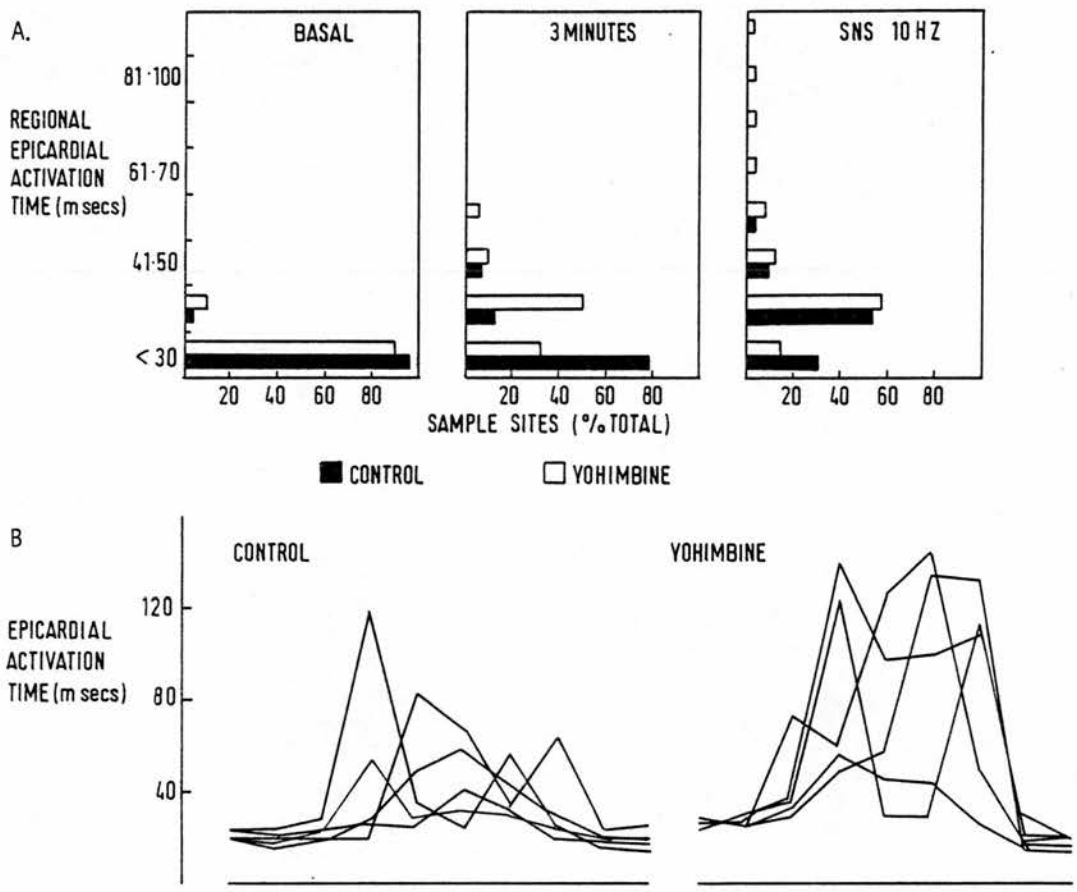


Figure 5.6 A Patterns of epicardial activation delay. Activation delay is plotted against number of electrodes with that delay for one experiment. Data are shown before and after yohimbine prior to and three minutes after coronary occlusion and during ansa stimulation. B Profiles of activation delay across the ischaemic zone from the same experiment. Data are shown from five rows of ten electrodes traversing the epicardium (ie 50 sites), recorded three minutes after coronary occlusion.

stimulaton with yohimbine but fibrillation did not occur with the control occlusion.

Group II - desmethylimipramine

The changes in regional myocardial catecholamine release, blood flow and lactate production after neuronal reuptake blockade were qualitatively similar to those following alpha$_2$-adrenoceptor inhibition.

Catecholamines:

[NA] release from the heart with ansa stimulation was potentiated across both ischaemic and non-ischaemic areas (Figure 5.7). Spontaneous [NA] release was not observed despite reuptake blockade. Expressed as the percentage increase in myocardial [NA] release following desmethylimipramine, it can be seen from figure 5.8 that at low frequency sympathetic stimulation and during reperfusion, the enhancement of release was greater across ischaemic compared to non-ischaemic myocardium. This suggests that neuronal reuptake was enhanced in ischaemic myocardium prior to drug administration.

Basal arterial [A] increased five fold following desmethylimipramine (from 0.3 $\pm$ 0.1 to 1.5 $\pm$ 0.7 pmol/ml; $p < 0.02$), the values rising parallel with those of the control period during ischaemia and reperfusion (Figure 5.9). Extraction of [A] was also maintained across both ischaemic and non-ischaemic myocardium during coronary occlusion with reuptake blockade, although extraction was diminished by ansa stimulation at 10 Hz

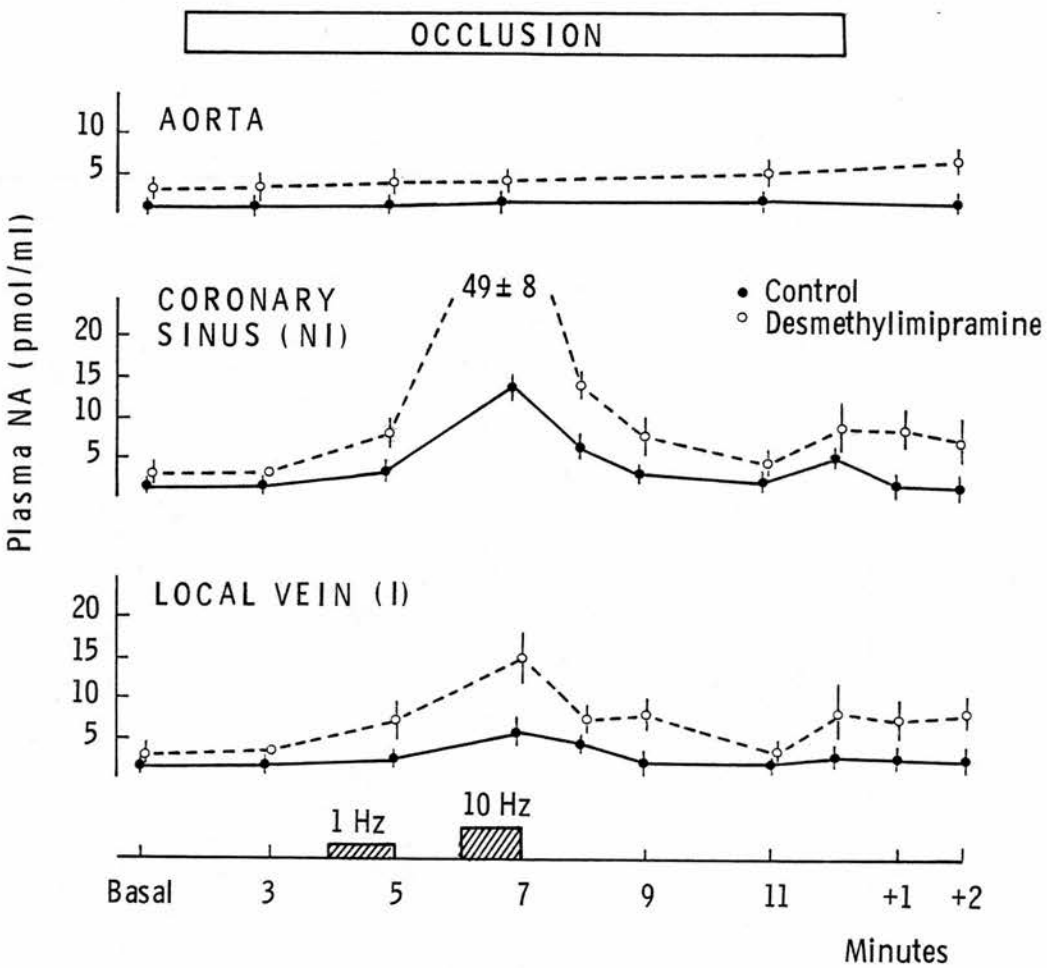


Figure 5.7 Plasma noradrenaline (NA) concentrations in arterial and in non-ischaemic and ischaemic venous effluent during coronary occlusion and reperfusion (mean ± SEM; n=6); effects of desmethylimipramine.

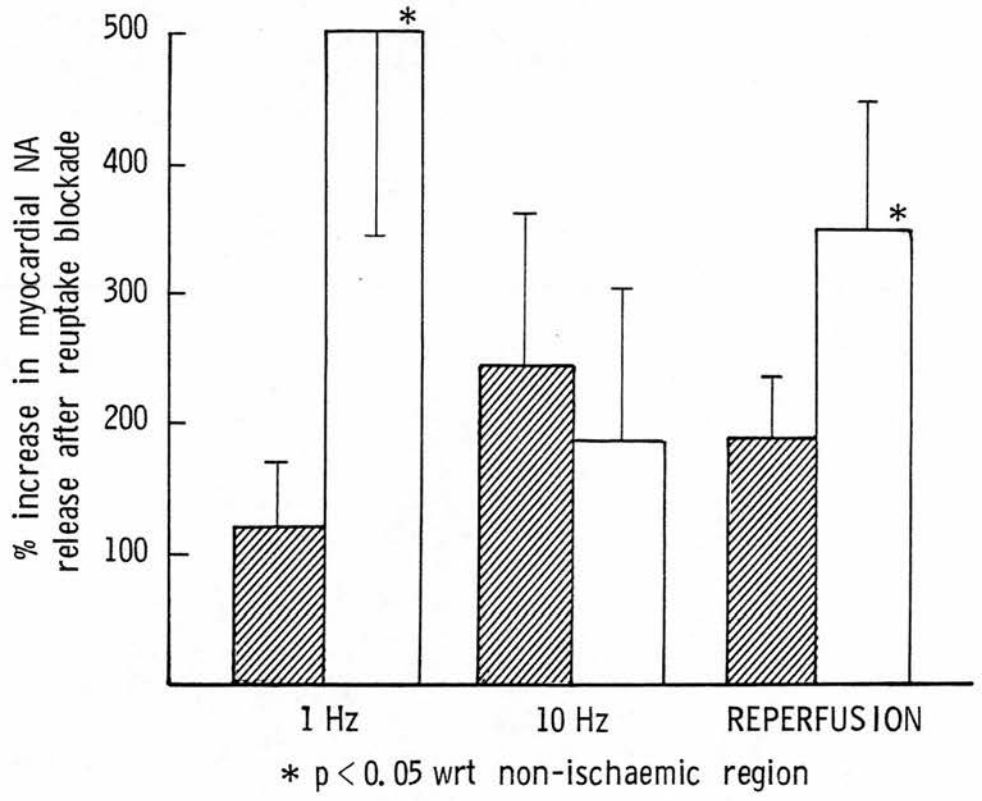


Figure 5.8 Increment in myocardial NA release from non-ischaemic (shaded bars) and ischaemic (open bars) myocardium following neuronal reuptake blockade with desmethylimipramine. Responses shown during low and high frequency ansa stimulation and during the first three minutes after coronary reperfusion.

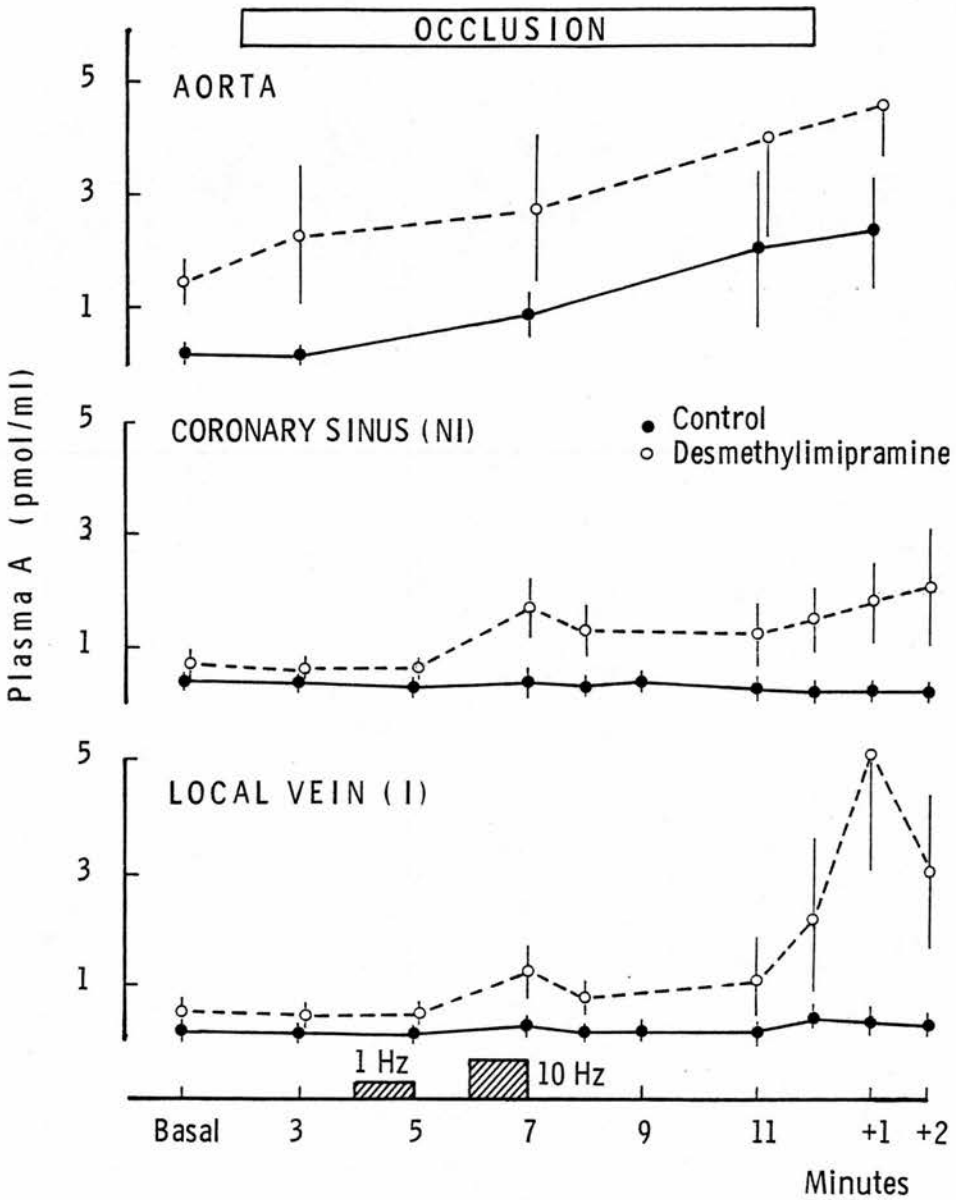


Figure 5.9 Plasma adrenaline (A) concentrations during coronary occlusion and reperfusion (mean ± SEM; n=6); effects of desmethylimipramine.

with desmethylimipramine. Reperfusion rapidly reduced [A] extraction across the previously ischaemic area, with minor and statistically insignificant release of [A] after one minute. No such release was observed during the control occlusion.

Lactate:

Myocardial lactate production across ischaemic myocardium occurred throughout occlusion with a statistically non-significant trend for increased production during sympathetic stimulation (figure 5.10). As noted after yohimbine (see Figure 5.5), extraction tended to fall across non-ischaemic myocardium during the period of coronary occlusion following reuptake blockade but the trend did not achieve statistical significance.

Haemodynamics:

Blood flow to non-ischaemic myocardium fell significantly after desmethylimipramine. Endocardial flow fell by 35 per cent from 115 $\pm$ 32 to 74 $\pm$ 32 ml/min/100 g ($p < 0.02$), while epicardial flow fell by 29 per cent from 114 $\pm$ 40 to 82 $\pm$ 40 ml/min/100 g ($p < 0.05$). An even greater reduction in flow was observed in the area of ischaemia, endocardial flow decreasing by 44 per cent from 35 $\pm$ 13 to 20 $\pm$ 15 ml/min/100 g and epicardial flow decreasing by 35 per cent from 44 $\pm$ 17 to 20 $\pm$ 25 ml/min/100 g ($p < 0.02$). During coronary occlusion, mean arterial blood pressure and pulse pressure did not change after reuptake blockade (108 $\pm$ 8 and 35 $\pm$ 9 mmHg control; 104 $\pm$ 18 and 32 $\pm$ 13 mmHg desmethylimipramine). The rises in mean pressure with low

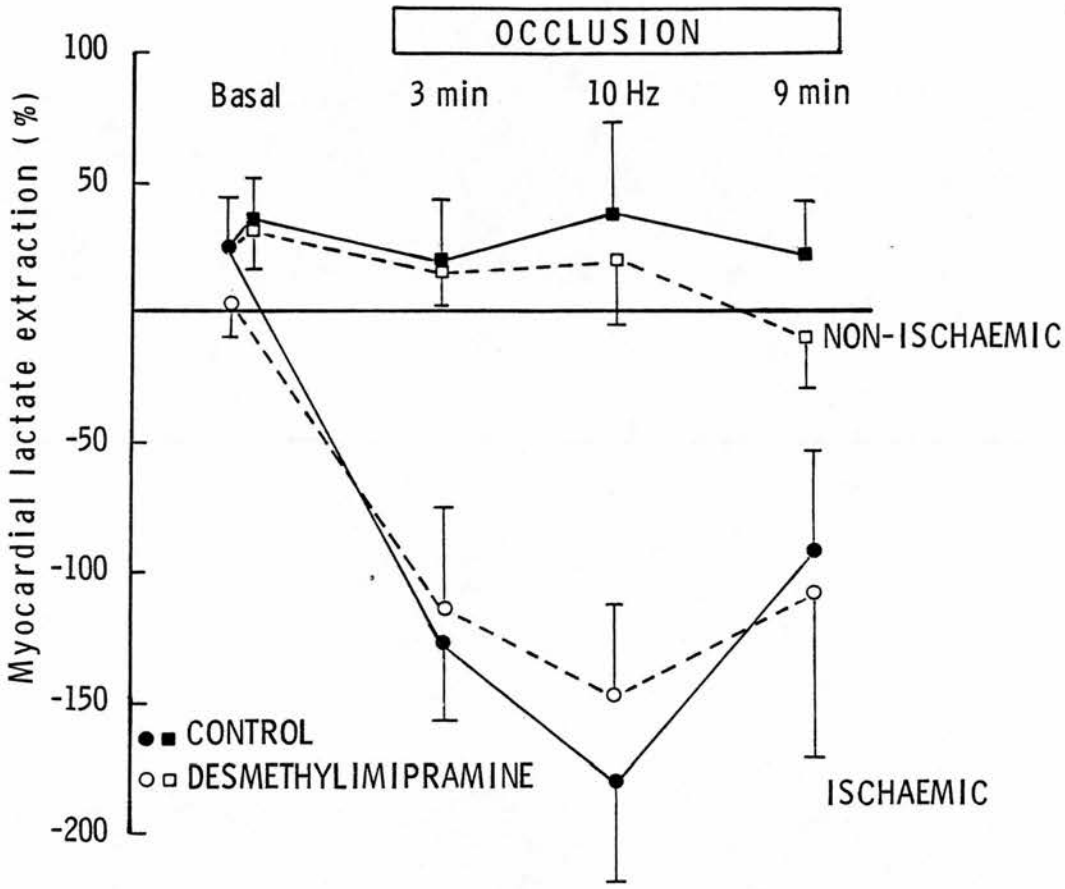


Figure 5.10 The effects of desmethylimipramine on myocardial lactate extraction (mean ± SEM; n=6). Data shown for samples withdrawn before occlusion, three and nine minutes after occlusion and immediately after high frequency ansa stimulation.

frequency ansa stimulation were similar (10 ± 11 mmHg control; 9 ± 15 mmHg desmethylimipramine), with high frequency the increment was larger after reuptake blockade (28 ± 16 mmHg control; 46 ± 21 mmHg desmethylimipramine; $p < 0.05$), possibly reflecting more intense myocardial catecholamine stimulation (Figure 5.7) at this time.

Electrophysiology:

Spontaneous ventricular fibrillation occurred once during control occlusions (17 per cent) and three times during occlusions after desmethylimipramine (50 per cent). The mean duration of coronary occlusion before fibrillation occured decreased with desmethylimipramine from 11 ± 1 to 9 ± 1 minutes ($0.05 < p < 0.1$).

Group III — Yohimbine - desmethylimipramine

Catecholamines:

In combination, yohimbine and desmethylimipramine resulted in marked increases in [NA] at all sampling sites (Figure 5.11), significantly greater than with either drug alone (Figures 5.2 and 5.7). Basal arterial [NA] rose 15 fold from 1.4 to 22.1 pmol/ml, although no significant spontaneous [NA] release from the heart was observed. During coronary occlusion, however, spontaneous release of [NA] from both ischaemic and non-ischaemic areas was observed, in contrast to control responses and that with yohimbine or desmethylimipramine alone (Figure 5.12). Release of [NA] during reperfusion occurred from the non-ischaemic as well as the

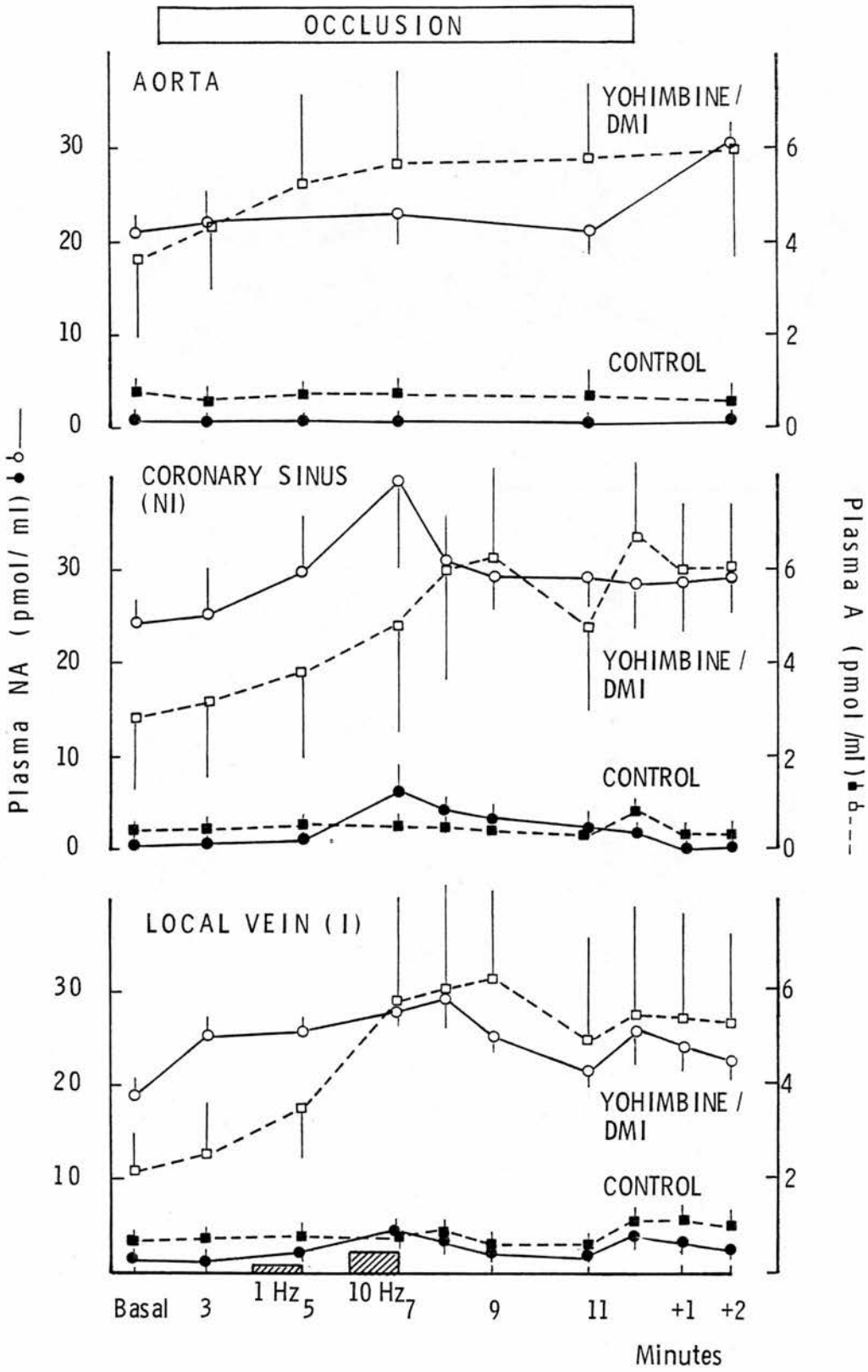


Figure 5.11 Plasma NA (circles) and A (squares) concentrations in arterial and in non-ischaemic and ischaemic venous effluent during coronary occlusion and reperfusion (mean ± SEM; n=7); effects of yohimbine and desmethylimipramine (DMI) in combination. Control data from Group I experiments.

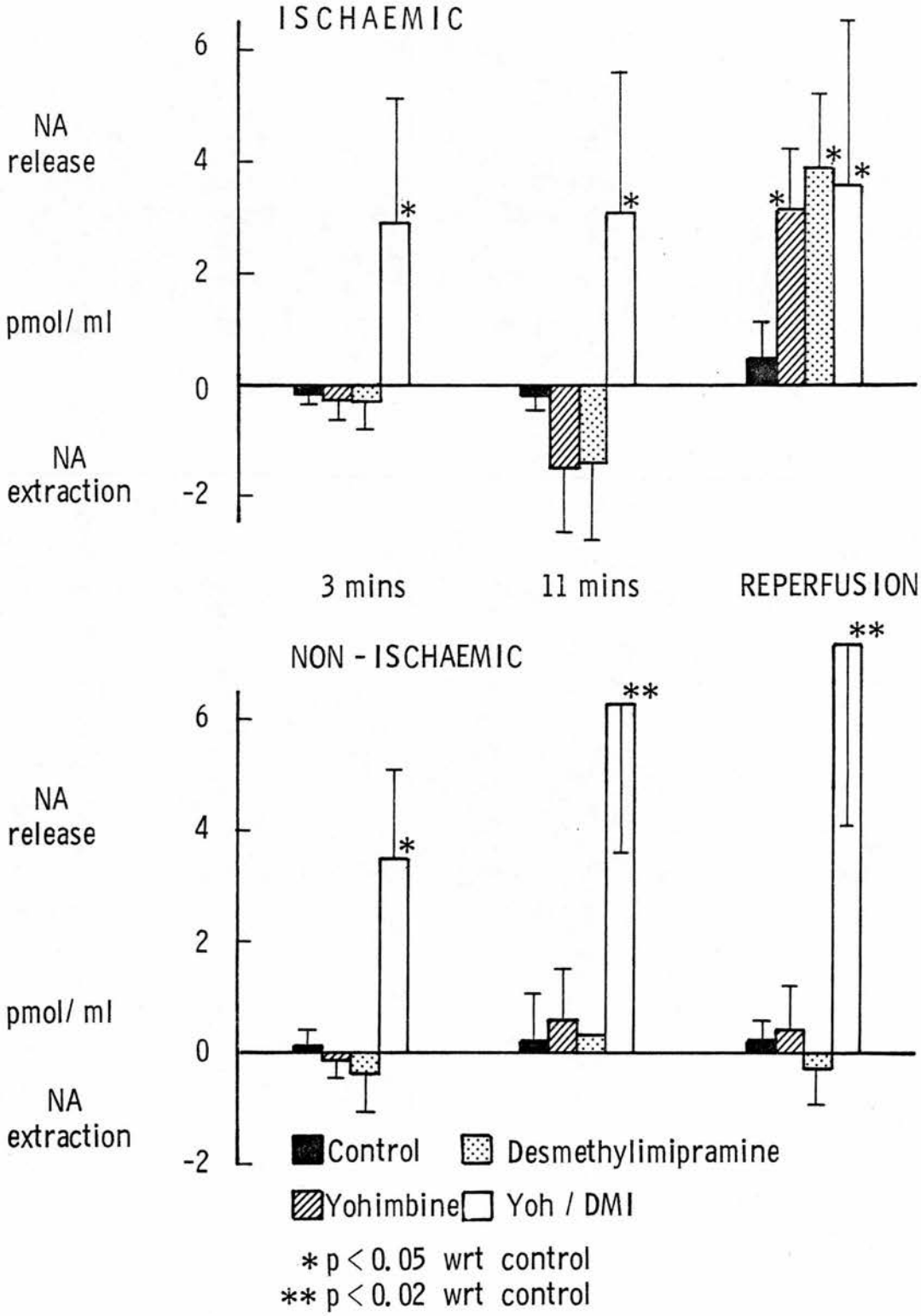


Figure 5.12 Spontaneous myocardial NA release/extraction from ischaemic and non-ischaemic areas during coronary occlusion and on reperfusion. Combined data from Groups I, II, and III.

ischaemic area, although the rapid rise in arterial [NA] at this time may have limited the accuracy of this observation. No further potentiation of enhanced reperfusion-induced overflow of [NA] from the ischaemic area was observed as a result of the drug combination. Surprisingly, increases in myocardial [NA] overflow with ansa stimulation were modest, especially from the ischaemic area (Figure 5.11). However, as spontaneous catecholamine release was observed prior to sympathetic stimulation, and as plasma levels of [NA] were so high, it may be that the capacity of the nerve terminals to release further transmitter as a result of additional direct nerve stimulation was limited. Despite this, however, epicardial activation abnormalities increased considerably during ansa stimulation with the drug combination (vide infra; Figure 5.14).

Arterial [A] was further increased with combined reuptake and $alpha_2$-adrenoceptor blockade (Figure 5.11) but the increase (1.5 $\pm$ 0.1 pmol/ml control; 3.7 $\pm$ 1.8 pmol/ml yohimbine/DMI) was less than that for [NA]. Extraction of [A] across the heart was maintained with the yohimbine/DMI combination both before (47 per cent control; 40 per cent yohimbine/DMI [p = NS]) and during (33 per cent control; 41 per cent yohimbine/DMI [p = NS]) coronary occlusion and was abolished during reperfusion. Spontaneous release of [A] was not observed.

Lactate:

As shown previously for both yohimbine and desmethylimipramine, myocardial lactate production occurred throughout the period of coronary occlusion and was restricted to

the ischaemic territory (Figure 5.13). Basal lactate extraction was reduced by yohimbine/DMI and production enhanced during maximal ansa stimulation and prior to reperfusion. Basal arterial lactate concentration increased from 1.2 ± 0.2 mmol/l to 2.1 ± 0.5 mmol/l ($p < 0.05$) with the drug combination.

Haemodynamics:

The small reduction in mean arterial blood pressure noted after yohimbine was maintained with the addition of desmethylimipramine (108 ± 9 mmHg control; 86 ± 5 mmHg yohimbine/DMI [$p < 0.05$]). The increase in mean pressure at low frequency ansa stimulation was unaffected (4 ± 4 mmHg control; 3 ± 4 mmHg yohimbine/DMI) while at high frequency, the increase was diminished (15 ± 13 mmHg control; 8 ± 5 mmHg yohimbine/DMI [$p < 0.05$]).

Electrophysiology:

Spontaneous ventricular fibrillation occurred once during control occlusions (14 per cent) and four times after yohimbine/DMI (57 per cent). The mean duration of coronary occlusion without ventricular fibrillation fell from 11 ± 1 to 7 ± 1 minutes ($p < 0.05$) with the drug combination.

Regional epicardial activation abnormalities after coronary occlusion were further intensified with combined reuptake/alpha$_2$-adrenoceptor blockade (Figure 5.14). At three minutes, mean activation delay increased by 59 per cent from 42 ± 2 to 67 ± 6 msecs ($p < 0.01$). At low frequency ansa stimulation,

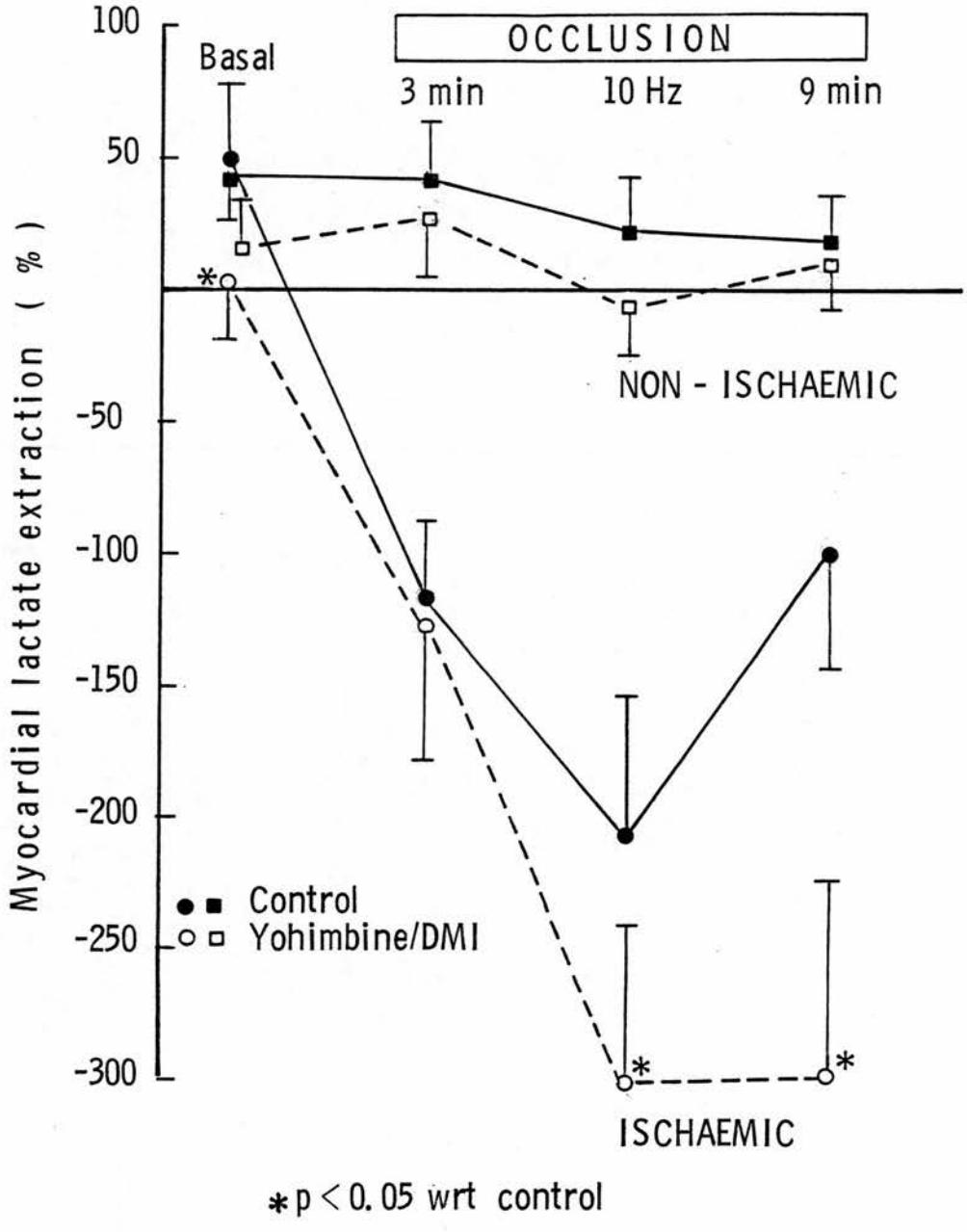


Figure 5.13 The effects of yohimbine/desmethylimipramine on myocardial lactate extraction (mean ± SEM; n=7).

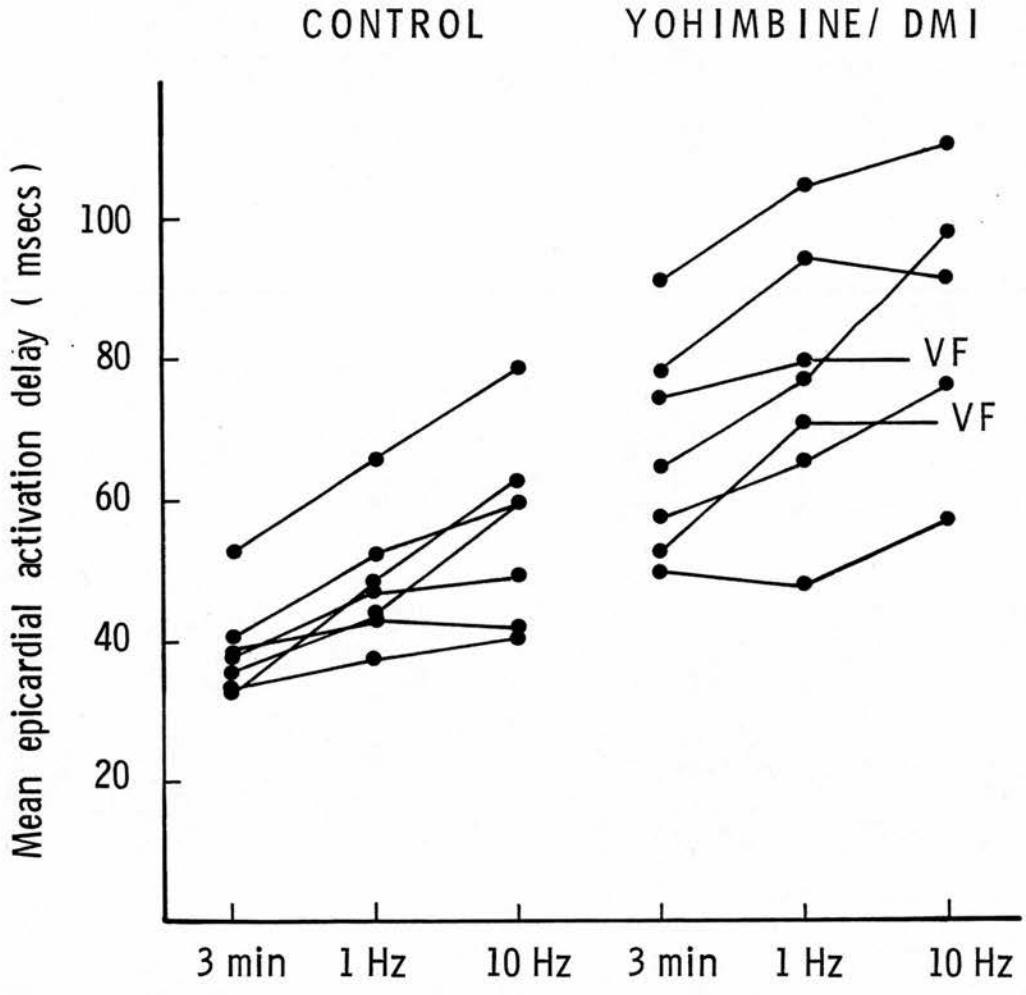


Figure 5 14 Mean epicardial activation delay before and after sym - pathetic stimulation within the central ischaemic area (n= 7); effects of yohimbine/desmethylimipramine. Ventricular fibrillation developed during high frequency ansa stimulation in two studies after yohimbine/ DMI.

activation delay increased from 48 ± 3 to 77 ± 8 msecs ($p < 0.01$) and at high frequency stimulation, activation delay increased from 56 ± 5 to 87 ± 9 msecs ($p < 0.01$).

Patterns of activation abnormality from one representative experiment are illustrated in Figure 5.15. Both the absolute levels and heterogeneity of activation times have been substantially increased by yohimbine/DMI, in keeping with an increase in spontaneous arrhythmias.

Group IV - Viloxazine

Noradrenaline extraction:

Before ischaemia, 1-^{3}H-NA extraction was similar across the anterior and lateral surfaces of the heart (42 ± 6 and 44 ± 8 per cent respectively). During coronary occlusion, extraction increased across the ischaemic area (51 ± 7 per cent; $p < 0.05$) but remained unchanged across the non-ischaemic area (47 ± 6 per cent) as shown in Table 5.3. Viloxazine resulted in a significant reduction in myocardial 1-^{3}H-NA extraction across both territories (51 ± 7 to 39 ± 5 per cent ischaemic; 47 ± 6 to 26 ± 5 per cent non-ischaemic; Table 5.3) but the percentage reduction in extraction was significantly greater across the non-ischaemic (45 ± 8 per cent) compared to the ischaemic (20 ± 4 per cent) area. Thus, despite enhanced 1-^{3}H-NA extraction across ischaemic myocardium, neuronal reuptake blockade with this drug results in a relatively small reduction in extraction at this site. It appears that this phenomenon of reduced activity of neuronal reuptake blocking drugs in ischaemic myocardium is not specific for viloxazine, since in a further three experiments using the same

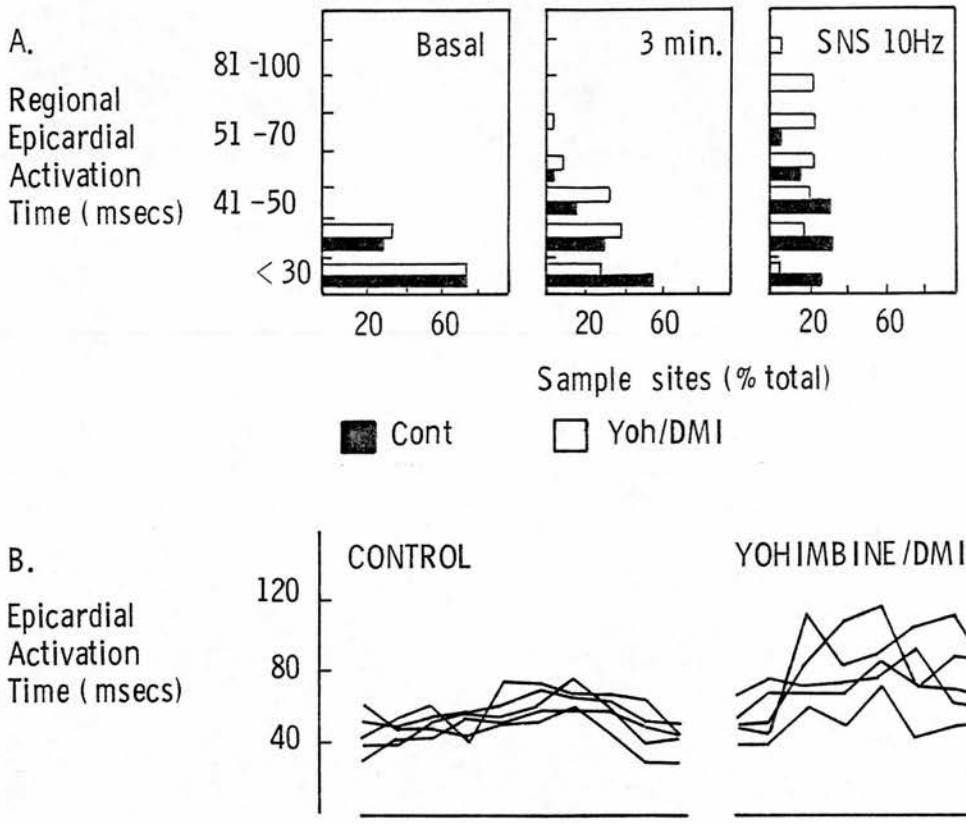


Figure 5.15 Patterns of epicardial activation delay; effects of yohimbine/DMI. A. Activation delay is plotted against number of electrodes with that delay before occlusion, three minutes after occlusion and during ansa stimulation. B. Profiles of activation delay across the ischaemic zone (same experiment). Data taken from five rows of ten electrodes across the epicardium (50 sites) recorded immediately after ansa stimulation (10 Hz).

EXPERIMENT	MYOCARDIAL 1-^{3}H-NORADRENALINE EXTRACTION (%)			
	ISCHAEMIC		NON-ISCHAEMIC	
	CONTROL	VILOXAZINE	CONTROL	VILOXAZINE
1	61	45	51	39
2	53	42	61	30
3	75	--	57	--
4	46	45	49	26
5	72	65	74	46
6	68	45	58	32
7	41	35	37	9
8	21	18	19	6
9	21	18	18	16
Mean ± SEM	51 ± 7	39^{+} ± 5	47 ± 6	26^{++} ± 5
	CONTROL I	CONTROL II	CONTROL I	CONTROL II
10	42	47	47	41
11	68	73	85	89
12	64	49	58	53
Mean ± SEM	58 ± 8	56 ± 9	63 ± 12	61 ± 14

$^{+}$ $p < 0.02$ wrt control $\quad$ $^{++}p < 0.001$ wrt control

Table 5.3: The effect of low dose (expts 1-6) and high dose (expts 7-9) viloxazine on extraction of 1-H^{3} noradrenaline across ischaemic and non-ischaemic myocardium. Mean extraction shown for each area during coronary occlusion. Expts 10-12 controls.

protocol, the percentage reduction in myocardial 1-^{3}H-NA extraction after desmethylimipramine (2.5 mg/kg) was similarly greater across the non-ischaemic (48 ± 10 per cent) compared to the ischaemic (34 ± 11 per cent) territory.

An example of the effect of high dose viloxazine on myocardial 1-^{3}H-NA extraction is shown in Figure 5.16. During the control occlusion (Figure 5.16a), extraction was increased slightly across the ischaemic compared to the non-ischaemic area with selective abolition of extraction across the ischaemic area on reperfusion (due to [NA] release). Arteriovenous differences were substantially reduced (although not abolished) by viloxazine (Figure 5.16b) but during coronary occlusion, extraction across the ischaemic area was greater than that across the non-ischaemic area. Reperfusion-induced noradrenaline release was readily observed from the previously ischaemic territory.

Lactate:

Lactate production during coronary occlusion was restricted to the ischaemic area. No significant differences in lactate release were noted between control occlusions and those after viloxazine. Before occlusion, lactate extraction was 37 ± 10 per cent before and 32 ± 6 per cent after viloxazine. Extraction fell to -156 ± 40 per cent during the control occlusion and -117 ± 38 per cent during occlusion after viloxazine.

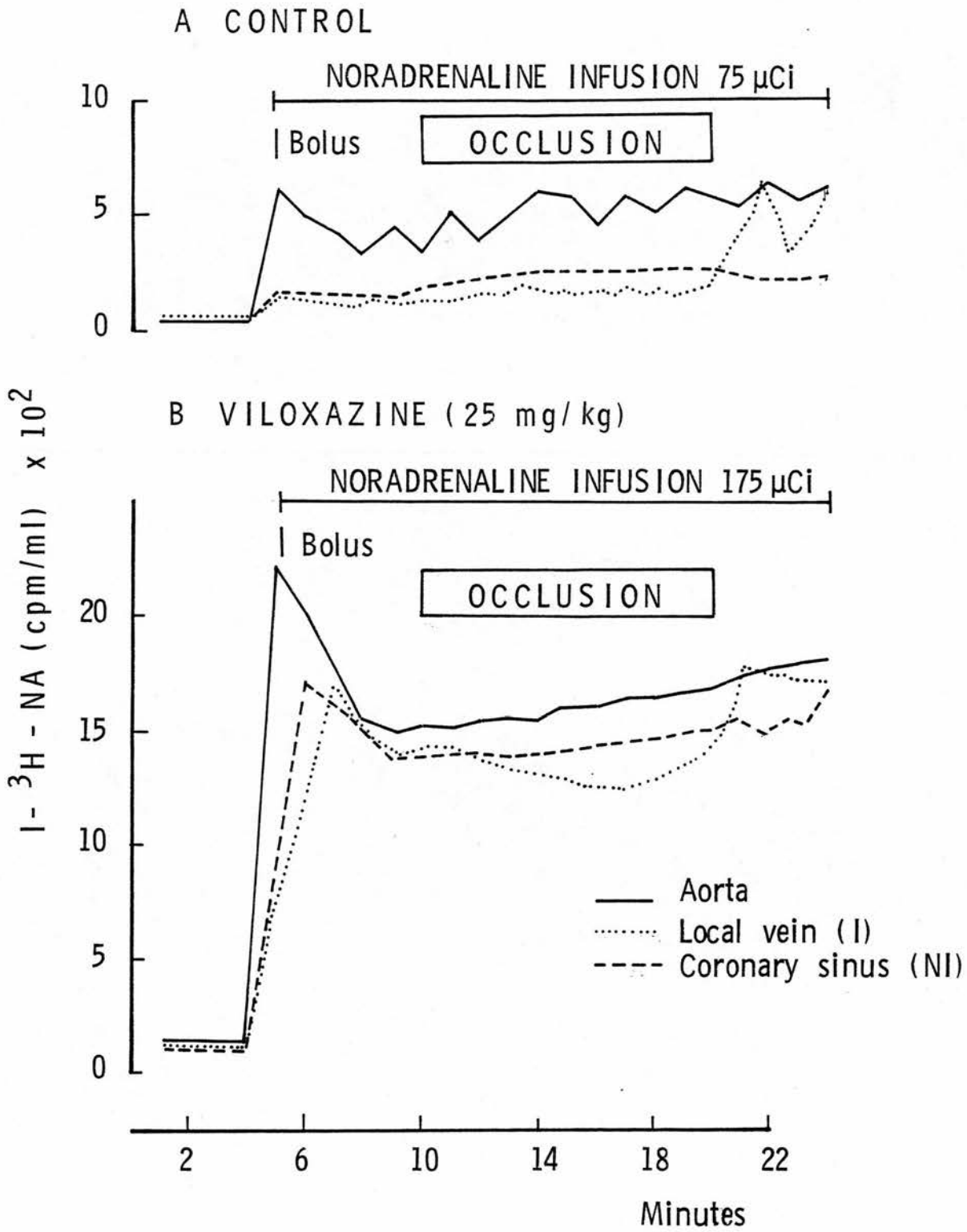


Figure 5.16 Myocardial NA extraction during continuous labelling with I-^{3}H-NA; effects of viloxazine (expt. 7).

Haemodynamics:

As with desmethylimipramine, blood flow to non-ischaemic myocardium fell after viloxazine. Endocardial flow fell by 26 per cent from 105 ± 11 to 78 ± 7 ml/min/100 g while epicardial flow fell by 25 per cent from 94 ± 8 to 71 ± 7 ml/min/100 g ($p < 0.05$ for both). The reduction in flow to the ischaemic area following viloxazine was similar, endocardial flow decreasing by 27 per cent from 30 ± 7 to 22 ± 4 ml/min/100 g and epicardial flow decreasing by 23 per cent from 40 ± 10 to 31 ± 8 ml/min/100 g ($0.05 < p < 0.1$). Mean arterial blood pressure fell from 103 ± 12 to 90 ± 9 mmHg after reuptake blockade.

Electrophysiology:

As noted previously for both yohimbine and desmethylimipramine singly and in combination, ventricular arrhythmias were more prevalent after reuptake blockade with viloxazine. Spontaneous ventricular fibrillation occurred twice during the control occlusion (22 per cent) and on four occasions (44 per cent) after viloxazine. The mean duration of coronary occlusion prior to ventricular fibrillation fell from 9 ± 1 to 7 ± 1 minutes ($0.05 < p < 0.1$).

The incidence of ventricular arrhythmias and duration of occlusion without ventricular fibrillation are summarised for each experimental group in Figure 5.17. The control data have been derived from all studies before drug administration The trend for enhanced arrhythmogenesis and reduced time to fibrillation is evident.

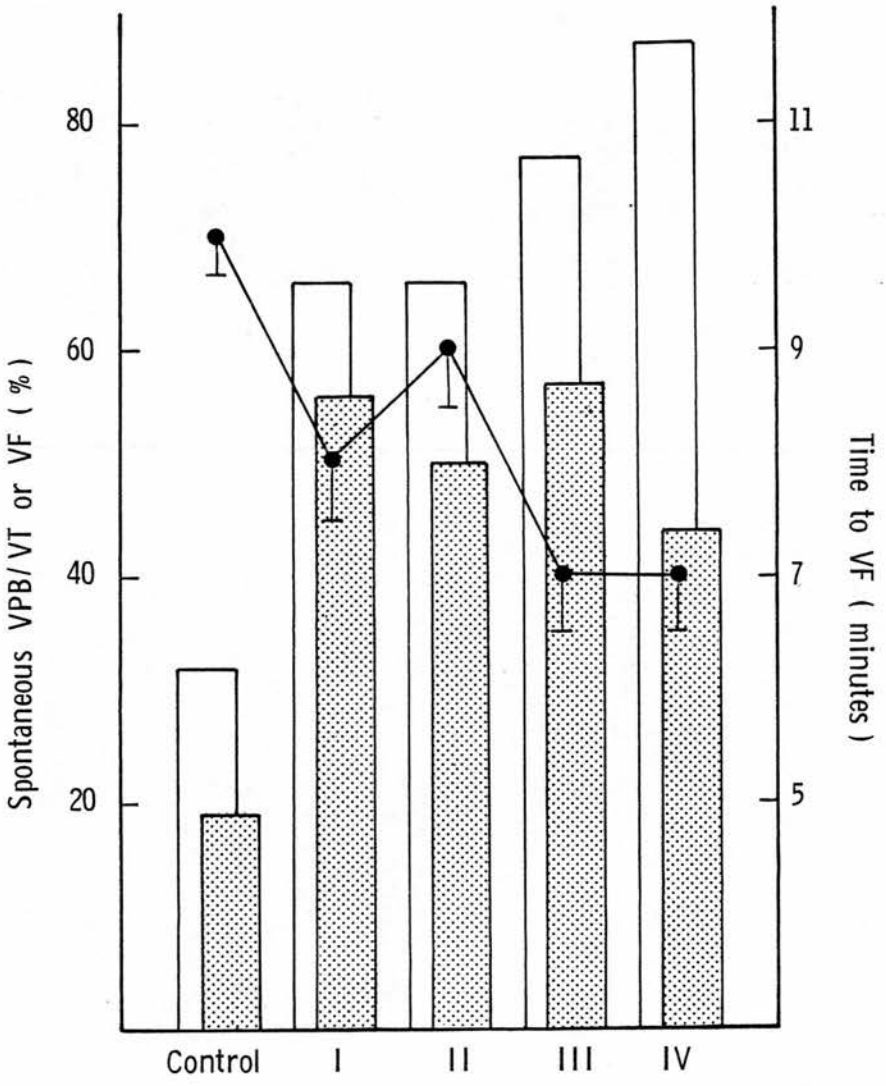


Figure 5.17 Percent incidence of ventricular tachycardia/ectopics (open histograms) or ventricular fibrillation (closed histograms) and duration of occlusion before ventricular fibrillation (closed circles); influence of presynaptic adrenoceptor modulation. control n=31; Gp I yohimbine n=9; Gp II desmethylimipramine n=6; Gp III yohimbine/desmethylimipramine n=7; Gp IV viloxazine n=9.

DISCUSSION

These studies indicate the importance of presynaptic adrenergic mechanisms in controlling catecholamine overflow from ischaemic and non-ischaemic myocardium and determining electrophysiological abnormalities during coronary occlusion. Furthermore, a relationship has been shown between the extent of potentiation of myocardial catecholamine release as a result of drug administration and epicardial activation abnormalities and spontaneous ventricular arrhythmias, such that the greater the potentiation of [NA] release, the greater the likelihood of arrhythmogenesis.

An alpha-adrenoceptor blocking agent with selectivity for the $alpha_2$-subtype, such as yohimbine, acts preferentially at the presynaptic level to block alpha-adrenoceptor-mediated negative feedback and thus increases local [NA] release (Tanaka and Starke, 1980). Although presynaptic $alpha_1$-adrenoceptor antagonists may reduce the early mortality from ventricular fibrillation during both circumflex (Manning and Caudwell, 1947) and LAD coronary occlusion (Sheridan et al, 1980), it is probable that the major influence of sympathetic stimulation on vulnerability to ventricular arrhythmias is mediated by beta-adrenoceptors (Malliani et al, 1980). Beta-adrenoceptor blockade substantially reduces enhanced vulnerability to ventricular arrhythmias associated with stellate ganglion stimulation, hypothalamic stimulation and catecholamine infusion (Lown et al, 1977). Thus, enhanced regional catecholamine release, secondary to presynaptic $alpha_2$-adrenoceptor blockade may be arrhythmogenic from increased beta-adrenoceptor stimulation, despite some postsynaptic $alpha_1$-adrenoceptor

blockade, evidenced in the yohimbine studies by a small fall in systolic blood pressure. Yohimbine increased arrhythmias and conduction abnormalities, and reduced blood flow to the ischaemic area during LAD occlusion in the anaesthetised dog model. Although some alpha-adrenoceptor blocking agents can modify [NA] release centrally, inhibit neuronal reuptake, and exhibit quinidine like effects on canine Purkinje fibres (Rosen et al, 1971), it is unlikely that yohimbine would have such activity in the dose selected for this study.

It is probable that a component of the increase in arterial catecholamines after yohimbine was secondary to a reflex increase in sympathetic tone from the fall in systemic blood pressure. This may be the explanation for the rise in arterial [A] with this drug (Figure 5.4), a factor that, theoretically, may further potentiate release of [NA] by activation of presynaptic beta-adrenoceptors. However, the rise in arterial [NA] was small compared to increases in myocardial [NA] release with sympathetic stimulation (Figure 5.3), the latter a direct assessment of the blocking action of yohimbine on nerve terminal adrenoceptors in the heart. Furthermore, it is myocardial, rather than systemic catecholamine release that is particularly associated with malignant arrhythmias (Hoffman, 1978). The three-fold increase in [NA] release with ansa stimulation after yohimbine demonstrates the activity of nerve terminal feedback mechanisms in acutely ischaemic myocardium. Indeed, [NA] release was increased more across the ischaemic (168 per cent rise) than across the non-ischaemic (86 per cent rise) territory. The [NA] concentration at the synaptic cleft is not readily predictable but will be many times greater than the change in ischaemic effluent.

Yohimbine increased both the severity and heterogeneity of epicardial activation abnormalities across the ischaemic area before and during sympathetic stimulation. Marked regional disparities in activation delay may result from temporal dispersion of epicardial activation with adjacent areas showing variable recovery of conduction, a situation predisposing to re-entry (Wit et al, 1974). Increased local [NA] concentrations may be arrhythmogenic through enhancement of slow-response action potentials, provocation of after-depolarisation or other mechanisms facilitating re-entry (Hoffman, 1978).

The selective reduction in RMBF to the ischaemic area with yohimbine would be compatible with an increase in coronary vascular resistance with this drug, although coronary perfusion pressure would have decreased with the fall in arterial blood pressure. Autoregulation presumably prevented a reduction in blood flow to the non-ischaemic area. After reuptake blockade with desmethylimipramine, however, RMBF was significantly reduced to both the non-ischaemic and particularly the ischaemic area, despite absence of change in blood pressure. It is quite possible, therefore, that increased myocardial [NA] release after either $alpha_2$-adrenoceptor blockade or reuptake blockade may increase coronary vascular tone through post-synaptic $alpha_1$-adrenoceptor activation and prevent maximal vasodilation secondary to accumulation of ischaemic vasodilator metabolites. Tonic alpha-adrenoceptor vasoconstrictor tone has been demonstrated in the normal coronary circulation (Feigl, 1975; Kelley and Feigl, 1978; Orlick et al, 1978; Murray and Vatner, 1979)- although controversy remains (Chilian et al, 1981)- and in the presence of a fixed coronary stenosis (Buffington and Feigl, 1981). Indeed, in

the presence of a severe circumflex coronary artery stenosis, Heusch and Deussen (1983) showed lactate production, arrhythmias and an increase in coronary vascular resistance distal to the stenosis as a result of cardiac sympathetic nerve stimulation. A reduction in coronary vascular resistance followed sympathetic stimulation when there was moderate or no circumflex stenosis. The increase in coronary vascular resistance was prevented by phentolamine. Stimulation of cardiac sympathetic nerves may also limit epicardial blood flow during maximal adenosine-induced metabolic vasodilatation (Johannsen et al, 1982). The reported here study supports the concept of alpha-adrenoceptor mediated coronary vasoconstriction as an inducer or intensifier of myocardial ischaemia distal to a severe coronary obstruction.

A direct effect of yohimbine or desmethylimipramine on coronary vasomotor tone cannot be excluded but is unlikely in view of their known pharmacological activity.

The potentiation of myocardial catecholamine overflow by desmethylimipramine was rather similar to that with yohimbine, although the rise in arterial [NA] was less marked. The latter observation may be explicable by absence of change in blood pressure with desmethylimipramine and hence no reflex increase in central neurosympathetic outflow. Expressed as the increase in myocardial [NA] release as a result of reuptake blockade with desmethylimipramine, it can be seen from Figure 5.8 that, at low frequency ansa stimulation, there is greater release across the ischaemic than the non-ischaemic area. This observation may be explained either by more effective reuptake blockade in the ischaemic area or enhanced reuptake activity at this site prior to

drug administration and hence greater drug efficacy. As shown in the viloxazine studies (Table 5.3) and in three studies with desmethylimipramine, extraction of 1-^{3}H-NA was less effectively reduced across the ischaemic, compared to the non-ischaemic area. Thus, despite less effective inhibition of neuronal reuptake, [NA] release was potentiated more across the ischaemic than the non-ischaemic area, strongly favouring enhanced reuptake at the former site i.e. before desmethylimipramine or viloxazine were given. No such differences were evident during high frequency ansa stimulation, possibly because factors other than neuronal reuptake, such as diffusion, washout or extraneuronal uptake may have determined removal of released neurotransmitter at such high and unphysiological concentrations. The activity of neuronal reuptake in limiting [NA] overflow from isolated perfused rat heart has recently been demonstrated (Schomig et al, 1983). Enhanced neuronal reuptake in acutely ischaemic myocardium and enhanced presynaptic negative feedback may be an explanation for absence of spontaneous [NA] overflow from the heart.

Flow itself is an important determinant of reuptake. A reduction in flow will reduce the concentration gradient between the juxtasynaptic and vascular spaces and hence reduce removal of released neurotransmitter by diffusion, allowing enhanced local reuptake, provided such processes remain active. In the isolated rat heart, [NA] extraction is enhanced during severe ischaemia (0.1 ml/min) but not with milder reductions in flow (3.0 ml/min) (Riemersma and Jynge, unpublished data). During low flow ischaemia, (0.05 ml/min/g beyond ten minutes) Dietz et al (1982) noted a three-fold increase in [NA] release from isolated perfused rat hearts during reperfusion in the presence of 100 μM cocaine.

With no flow ischaemia, no such potentiation of [NA] release with uptake blockade was evident. Thus, complete cessation of flow may inhibit neuronal reuptake, an energy dependent process. It has also been suggested that the massaging action of the heart may aid diffusion of released neurotransmitter into the blood stream (Levy 1982). This mechanism will be reduced or absent in the ischaemic territory. The control studies in group IV revealed enhanced extraction of 1-^{3}H-NA across the ischaemic territory during coronary occlusion. Increased extraction at this site was maintained after viloxazine, despite, as shown in Table 5.3, reduced efficacy of this reuptake blocker in ischaemic myocardium.

The importance of extraneuronal uptake in removal of released [NA] has not been assessed in these studies although catecholamine metabolites may have contributed to tritium efflux in group IV. It is likely, however, that extraneuronal uptake is much less important than neuronal uptake in removing released neurotransmitter from sympathetic nerve terminals, since extraneuronal uptake blockade has little influence on the magnitude and duration of the chronotropic and inotropic response to sympathetic stimulation (Matsuda et al, 1979). In contrast, neuronal uptake blockade has a pronounced effect on the duration of the chronotropic and to a lesser extent the inotropic response (Koerker and Moran, 1971; Levy and Blattberg, 1978; Matsuda et al, 1980).

Both yohimbine and desmethylimipramine potentiated reperfusion-induced [NA] release from the ischaemic area. As discussed in Chapters 3 and 4, it seems more likely that this release is specific to reperfusion per se, rather than washout of

[NA] released during the period of ischaemia and if this is so, it is probable that the transmitter is being released from nerve terminals in the ischaemic zone. Reperfusion-induced ventricular fibrillation occurred twice as frequently after reuptake or alpha-adrenoceptor blockade as in control studies.

Given in combination, yohimbine and desmethylimipramine caused marked increases in basal catecholamine concentrations at all sampling sites. Release of [NA] from the heart was not observed, under basal conditions. At both three and eleven minutes after coronary occlusion, however, spontaneous release of [NA] from ischaemic and non-ischaemic areas was observed, contrasting with responses to either drug given alone and to the control occlusion. Even under these conditions of combined $alpha_2$-adrenoceptor and neuronal reuptake blockade, the increase in myocardial [NA] release as a result of coronary occlusion, while statistically significant, represented a modest rise over substantially elevated arterial levels (Figures 5.11 and 5.12). Using this model, therefore, spontaneous release of [NA] from the heart during coronary occlusion can be unmasked only by attenuation of neuronal reuptake and $alpha_2$-adrenoceptor mediated negative feedback mechanisms. Neither agent caused spontaneous release of [NA] from the heart when used alone, presumably because of the efficacy of the alternative mechanisms for reducing [NA] spillover from the synaptic cleft, an effect recently shown by Enero (1983) for changes in rat atrial rate during sympathetic stimulation. The modest increase in myocardial [NA] release following ansa stimulation in the studies reported here may also partly reflect nerve fatigue as a result of intense drug-induced nerve terminal activity.

It is, perhaps, not surprising that epicardial activation abnormalities across the ischaemic area were further intensified by the drug combination (Figures 5.14), compared to yohimbine alone (Table 5.2). These findings are in keeping with the hypothesis that enhanced local myocardial catecholamine turnover increases electrophysiological abnormalities resulting from acute ischaemia and predisposes to arrhythmias based on re-entry.

Diminished activity (assessed by the reduction in 1-^{3}H-NA extraction) of both reuptake inhibitors in ischaemic as opposed to non-ischaemic myocardium was an unexpected finding. Drug delivery to both areas should have been the same. The mechanism by which diminished activity of reuptake blockers occurs in ischaemic tissue is unknown but may involve impaired access or binding to its receptor, or the presence of metabolites that impair signal transformation processes. The effect of calcium channel blockers on developed tension in cat papillary muscle, for example, alters significantly with change in pH (Briscoe and Smith, 1982). A relationship between the differential efficacy of reuptake inhibitory drugs in ischaemic and non-ischaemic myocardium (i.e. the difference in extraction across each area) and the likelihood of arrhythmias during coronary occlusion following viloxazine was suggested by the finding of a greater difference in regional extraction (48 $\pm$ 19 per cent; n = 5), in those experiments where arrhythmias were more severe or occurred earlier, than in those where arrhythmias did not increase (18 $\pm$ 12 per cent; n = 3; $p < 0.05$). Differential efficacy of reuptake inhibitory drugs across the heart will create a [NA] concentration gradient between ischaemic and non-ischaemic areas and may enhance the likelihood of arrhythmia production.

Extraction of [A] was maintained across both ischaemic and non-ischaemic areas and was not significantly reduced by yohimbine or DMI singly and in combination, suggesting that extraction is not dependent on either neuronal uptake or alpha$_2$-adrenoceptor mechanisms. It is probable that extraction of [A] across the heart is predominantly extraneuronal, a process known to have a higher affinity for [A] compared to [NA] (Iverson, 1967). Energy-dependent neuronal uptake of [A] in vascular tissue has, however, been described (Abrahamsen and Nedergaard, 1983). Although spontaneous release of [A] from the heart was not observed at any time, abolition of [A] extraction on reperfusion after DMI and DMI/yohimbine in combination does suggest some release of [A] from the reperfused area, as complete absence of extraction at this time is improbable. Release of [A] from the heart during reperfusion may potentiate [NA] release through its action at presynaptic beta-adrenoceptors. In this respect, [A] may act as a functional co-transmitter (Majewski et al, 1983).

Myocardial lactate production was restricted to the ischaemic area in all studies and was increased following sympathetic stimulation. Yohimbine and yohimbine/DMI in combination reduced lactate extraction prior to coronary occlusion and reduced extraction across the non-ischaemic area during coronary occlusion (Figures 5.5 and 5.13), but only in combination was release increased during ischaemia. An increase in myocardial lactate release with yohimbine/DMI may reflect enhanced catecholamine mediated stimulation of anaerobic glycolysis in the ischaemic zone.

This study has suggested enhanced activity both of neuronal reuptake and alpha-adrenoceptor negative feedback in nerve

terminals within acutely ischaemic myocardium. Both mechanisms act independently to limit myocardial catecholamine release and the consequent detrimental effects of released neurotransmitter on electrophysiological abnormalities and regional blood flow. The beneficial effects of post-synaptic alpha-adrenoceptor blockade in acute ischaemia may be offset by enhanced local catecholamine turnover. Prazosin, for example, appears not to increase catecholamine levels or myocardial catecholamine release in conscious dogs (Holtz and Bassenge, 1981). An alternative approach, worth further investigation, is the possible antiarrhythmic action of $alpha_2$-adrenoceptor agonists that may switch off local [NA] release through activation of presynaptic negative feedback. Preliminary data from three pilot experiments using clonidine are shown in Figure 5.18. It can be seen that clonidine acts as a powerful peripheral inhibitor of myocardial [NA] release in response to sympathetic stimulation. Basal [NA] at both arterial and venous sampling sites was also decreased by this drug and heart rate fell by 32, 46 and 52 beats per minute at the 1, 10 and 50 µg/kg doses respectively. Alternative agents without central sedative effects may be preferable for further evaluation.

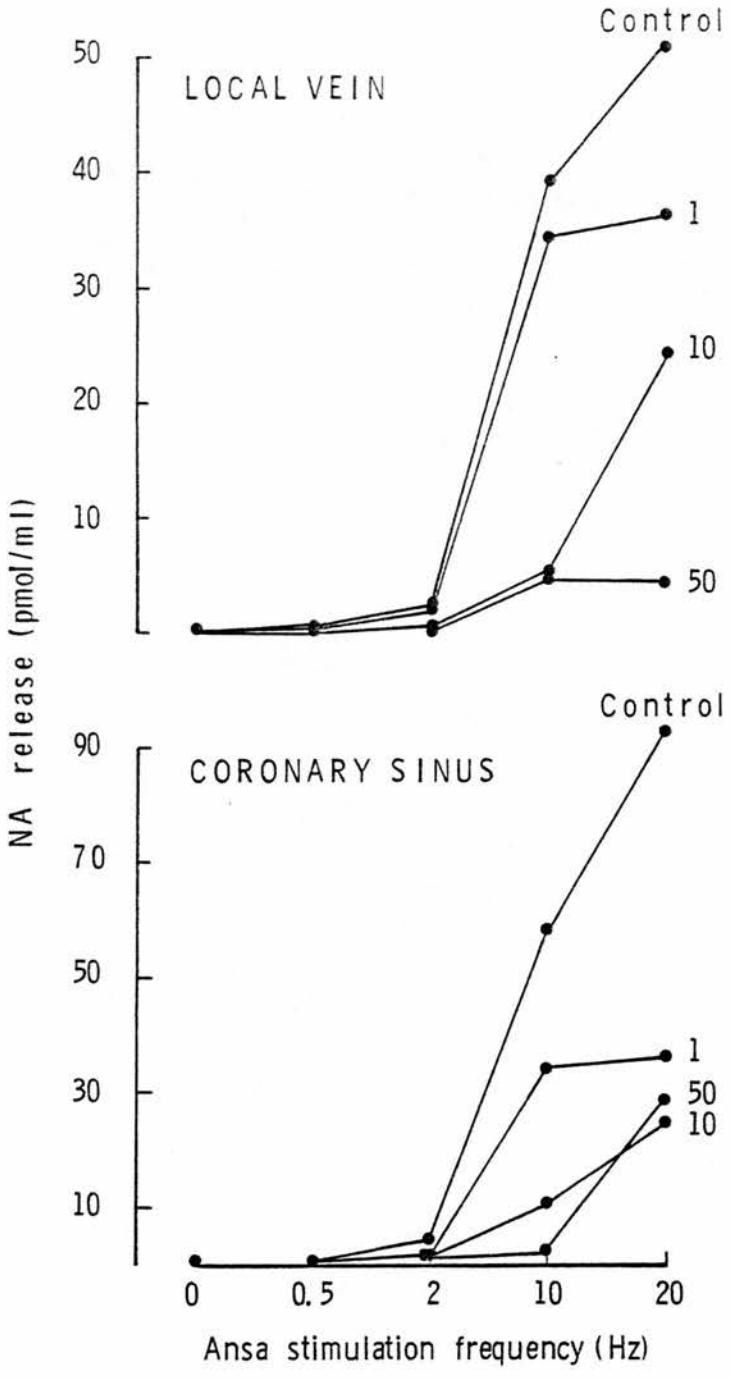


Figure 5.18 Myocardial NA release in response to sympathetic stimulation; effects of clonidine. The ansa subclavia from the left stellate ganglion was stimulated at the frequencies shown before and twenty minutes after intravenous clonidine at three doses - 1, 10 and 50 µg/kg (pentobarbitone anaesthetised open-chest dog; n=3).

6 METABOLIC MODULATION OF SYMPATHETIC NERVE TERMINAL ACTIVITY: EFFECTS OF REGIONAL HYPERKALAEMIA AND ADENOSINE

One of the earliest metabolic changes in the myocardium during coronary ligation is loss of intracellular potassium from ischaemic cells. Early attempts to quantify the secondary rise in extracellular potassium relied on measurement of coronary arteriovenous differences (Harris et al, 1958; Regan et al, 1967, 1969; Thomas et al, 1970; Ettinger et al, 1973), almost certainly a considerable underestimate of true changes in the interstitial space. More recently, however, the use of ion-sensitive electrodes has permitted direct measurement of extracellular potassium during myocardial ischaemia (Hill and Gettes, 1980; Hirsche et al, 1980; Weiss and Shine, 1981). Levels start to rise some ten seconds after coronary occlusion in the pig model and reach 15-20 mM in the central ischaemic zone within the first five minutes of ischaemia at the time of onset of early ventricular arrhythmias (Hirsche et al, 1980). At this concentration, important electrophysiological effects on the myocardium have been demonstrated, including inhibition of the fast inward sodium channel and shortening of the duration of the action potential (Opie et al, 1979).

In parallel with this evidence, it has been known for over a decade that potassium concentrations between 5 and 20 mM decrease the reactivity of blood vessels to sympathetic stimulation in vitro through inhibition of noradrenaline release while beyond 20 mM, the transmitter is released directly (Kirkepar et al, 1972; Lorenz and Vanhoutte, 1975).

The first part of the series presented in this chapter therefore examines in vivo myocardial release of noradrenaline in response to sympathetic stimulation during regional intracoronary infusions of potassium designed to mimic concentrations shown by

direct measurement during ischaemia. It is hypothesised that release of potassium from ischaemic myocardium may directly influence noradrenaline release from adjacent nerve terminals and hence indirectly modify both myocardial and coronary vascular reactivity.

Purine release from ischaemic myocardium, especially adenosine, occurs following coronary occlusion and the altered levels are paralled by altered flow (Olsson, 1970; Rubio et al, 1974; Olsson et al, 1978). Because adenosine is a potent vasodilator when injected into the coronary arteries (Drury and Szent-Gyorgyi, 1929) it has been suggested that it may play a role in mediating coronary vasodilatation during ischaemia and hypoxia and thus act as a determinant of regional myocardial perfusion.

In vitro, however, adenosine also modifies the response of canine vascular strips to sympathetic nerve stimulation (Verhaeghe et al, 1977). A dose-dependent inhibition of the contractile response to electrical stimulation occurs, associated with reduced efflux of noradrenaline during nerve stimulation. Vasodilatation during adenosine infusion into the lateral saphenous vein of the dog is also greater during vasoconstriction from electrical stimulation of sympathetic nerves than from exogenous noradrenaline. The effects of adenosine on myocardial noradrenaline release and coronary vascular resistance during sympathetic stimulation in-vivo have not been examined.

The second part of this study therefore examined whether basal or nerve stimulated myocardial noradrenaline release was modified by regional adenosine infusion and whether the effects of sympathetic stimulation on coronary vascular reactivity changed as

a result of adenosine-induced coronary vasodilation.

For both studies, potassium and adenosine were infused selectively into the LAD with venous sampling from a local coronary vein in this territory and in the territory of drainage of the circumflex coronary artery which acted as the control.

METHODS, PROTOCOLS

Adult mongrel dogs of either sex were anaesthetised with pentobarbitone and prepared surgically as described in Chapter 4 with two modifications:-

Firstly, a fine (1 FG) Portex cannula was inserted into a small proximal branch of the LAD, usually found running laterally round the root of the pulmonary artery towards the right ventricle. The artery (ext. diameter approximately 1-1.5 mm) was carefully dissected along its length to ensure that small branches were absent or ligated and the cannula inserted and advanced to the branch point with the LAD. Preliminary studies had shown that this method allowed infusion into the LAD without reducing flow in the parent artery. The length of cannula to be inserted was carefully measured against the branch vessel (usually 1-1.5 cm) and the position of the cannula tip checked at the end of each experiment. Selective infusion into the LAD was also confirmed at the end of the experimental preparation by observing a selective increase in LAD (but not circumflex) flow after infusion of 100 µl 10^{-4} M adenosine. The position of the cannula was, if necessary, adjusted and rechecked.

Secondly, both the circumflex and LAD were dissected over

approximately one centimetre within three centimetres of their origin and electromagnetic flow probes (internal diameter 2-3 mm) placed round each vessel for continuous measurement of flow using a two-channel flowmeter (Skalar Instruments). The flowmeter zero in isotonic saline at 37°C was checked at intervals during each experiment. Zero-drift characteristics were within specification and checked and if necessary adjusted hourly.

Heart rate was kept constant throughout each experiment by atrial pacing at a rate of 5-10 beats per minute faster than the maximum rate during ansa stimulation (the paced rate varied between 142 and 204 beats per minute) but was kept constant throughout each experiment.

Thirty minutes after completion of the experimental preparation, intracoronary infusions of isotonic saline or potassium chloride (10, 25, 75 mM; first series) and isotonic saline or adenosine (10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} M; second series) were established at a rate less than 5 per cent of measured LAD flow and continued for thirty minutes. The infusion rate of potassium was adjusted on the basis of the measured basal LAD flow to achieve an arterial K^+ level of +1, +2 and +7.5 mM for each study but was thereafter kept constant. K^+ infusions above 150 mM tended to cause progressive LAD vasoconstriction preventing the establishment of a steady state. Blood pressure, LAD and circumflex flow were measured continuously. After 10 minutes, changes in mean flow, aortic diastolic pressure and regional myocardial [NA] release from the anterior (LAD) and lateral (circumflex) surfaces of the heart were evaluated during graded stimulation of the ansa subclavia from the left stellate ganglion at supramaximal voltage (8-10 v; 4

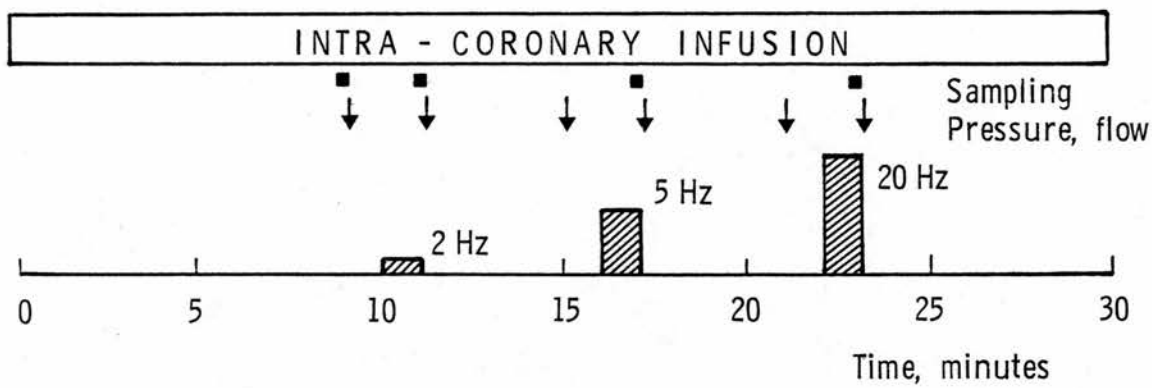


Figure 6.1 Metabolic modulation of sympathetic responsiveness. Protocol for regional infusion studies (saline, potassium, adenosine; seetext for details) and graded ansa stimulation (2, 5, 20 Hz).
squares = arterial/venous sampling. arrows = aortic diastolic pressure and regional coronary flow measurement.

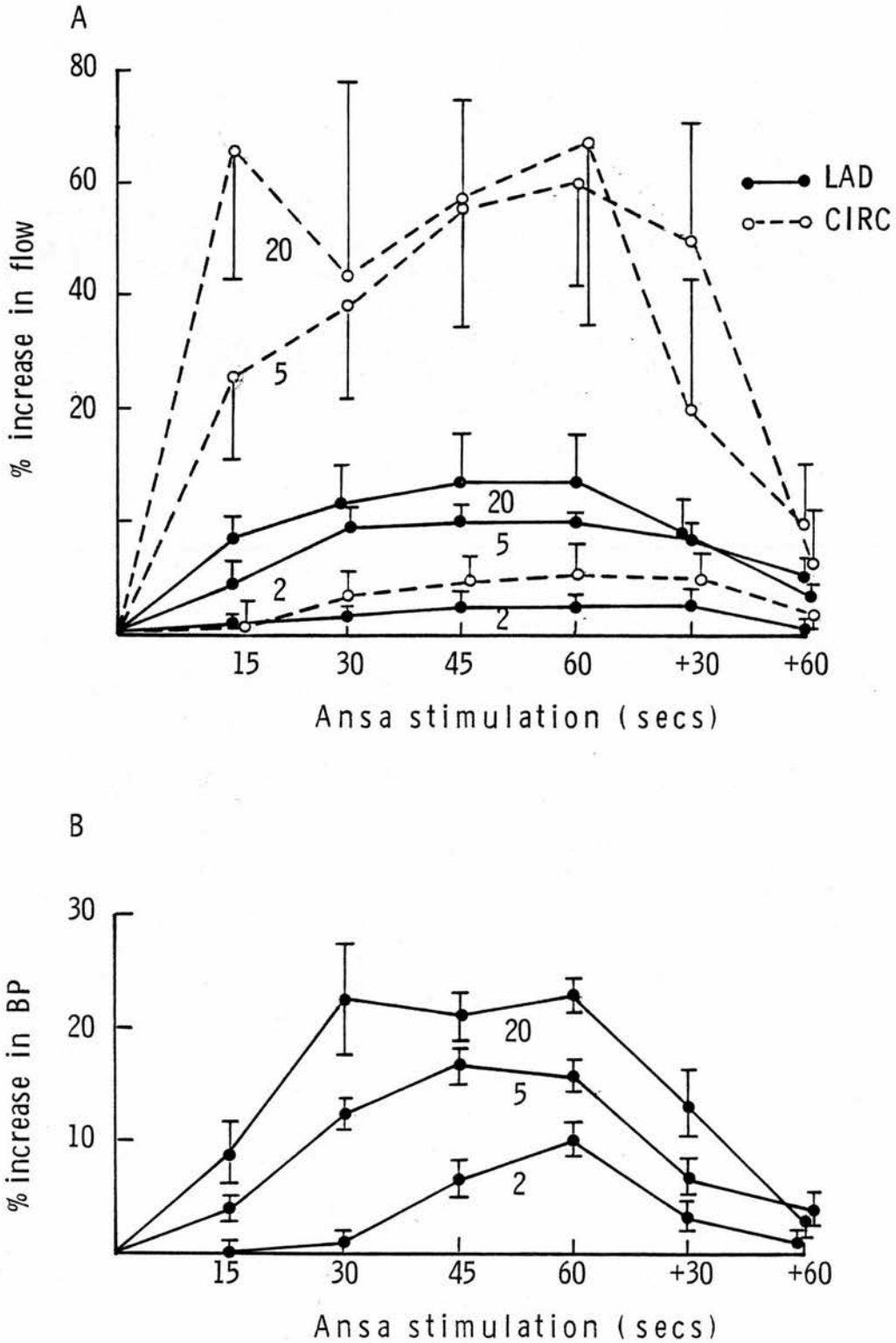


Figure 6.2 Haemodynamic responses (mean ± SEM) during and after graded ansa stimulation (2, 5, 20Hz). Increments in regional myocardial blood flow (A) and mean aortic diastolic blood pressure (B). Control experiments (n=6; 3 expts).

msecs) and varying frequency (2, 5, 20 Hz) as shown in Figure 6.1. A further twenty minute recovery period was allowed before the protocol was repeated. Coronary vascular resistance was calculated from

$$\frac{\text{aortic diastolic pressure (mmHg)}}{\text{mean flow (ml/min)}}$$

Arterial and coronary venous potassium levels were measured in the first series at the start and finish of each infusion period.

Changes in regional coronary blood flow and mean aortic diastolic pressure during six periods of graded ansa stimulation for one minute in three preliminary experiments are shown in Figure 6.2. Although there was considerable inter-experimental variability in the responses to sympathetic stimulation, in keeping with variable noradrenaline release, maximum responses were evident after one minute of stimulation, and this time period was therefore selected for this study. Flow increases in the circumflex coronary artery were greater than the LAD at all stimulation frequencies.

Each of the three potassium or four adenosine dose ranges were given in random order for each experiment with a saline control infusion before and at the end of each metabolite study. Control data have been calculated from the mean of these two saline responses.

All plasma samples were collected into pre-cooled tubes over 15 seconds before and during the final 15 seconds of ansa stimulation. Plasma was immediately separated and frozen for subsequent analysis in duplicate as described previously.

Differences in regional myocardial catecholamine release,

blood flow and coronary vascular resistence at each level of ansa stimulation, were evaluated by analysis of variance and computed modified t-statistic for pair differences. In view of the considerable inter-experimental variability in basal flow, haemodynamic data have been normalised to mean baseline measurements before and at the end of each sequence of ansa stimulation. All data are expressed as mean $\pm$ SEM. A five per cent level of confidence was considered statistically significant.

RESULTS

Series I: Potassium

Stimulation of the left ansa subclavia at increasing frequency resulted in progressive [NA] release from both anterior and lateral surfaces of the heart. Release was greater from the lateral (circumflex) territory at all levels of ansa stimulation as noted previously (at 20 Hz, [NA] release 10.2 $\pm$ 2.9 pmol/ml anterior; 21.2 $\pm$ 6.9 pmol/ml lateral; $p < 0.005$).

Spontaneous release of [NA] from the heart did not occur with intracoronary potassium infusion. During low dose potassium infusions (10 mmol/ml) despite a minor and statistically insignificant rise in anterior venous K^+, [NA] release was significantly reduced at both 5 and 20 Hz stimulation (peak release at 5 Hz 4.8 $\pm$ 1.1 pmol/ml control and 2.7 $\pm$ 0.8 pmol/ml 10 mM KCl, $p < 0.05$; peak release at 20 Hz 10.2 $\pm$ 2.6 pmol/ml control and 2.9 $\pm$ 1.8 pmol/ml 10 mM KCl, $p < 0.01$; Figure 6.3a). During 75 mM potassium infusion [NA] release was potentiated at high frequency stimulation (10.2 $\pm$ 2.6 pmol/ml control, 13.3 $\pm$ 2.7 pmol/ml 75 mM KCl, $p < 0.05$) when coronary venous K^+ increased from

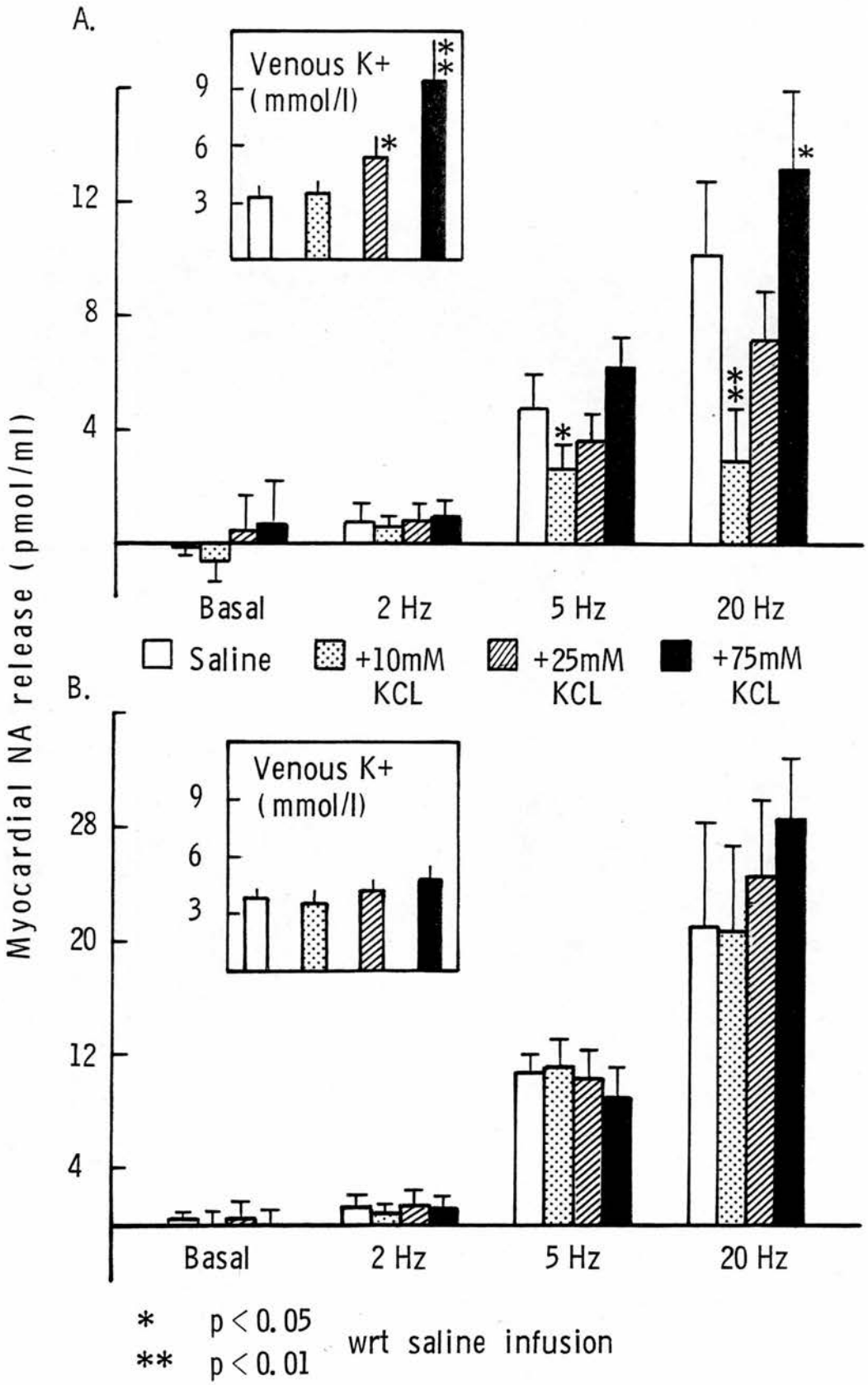


Figure 6.3 Myocardial NA release during graded ansa stimulation -effects of regional potassium infusion (n=6; mean ± SEM). Insets show venous K in anterior (LAD; A) and lateral (circumflex; B) veins.

3.6 ± .4 to 9.7 ± 3.4 mmol/l ($p < 0.01$). The trend for a biphasic action of potassium on regional [NA] release from the heart (inhibition at low dose and potentiation at high dose) was evident at all stimulation frequencies, as shown in Figure 6.3a. In contrast, no significant dose related differences in [NA] release from the lateral (circumflex) territory were noted during ansa stimulation. Coronary venous K^+ was not significantly increased at this site (Figure 6.3b).

Basal coronary artery flow was not significantly changed by intracoronary potassium at any of the three concentrations (46 ± 8 ml/min saline; 44 ± 9 ml/min 10 mM KCl; 42 ± 5 ml/min 25 mM KCl and 41 ± 4 ml/min 75 mM KCl). Similarly, arterial pressure remained constant. Changes in coronary flow and coronary vascular resistance during ansa stimulation and regional potassium infusion are summarised in Figure 6.4. Control studies showed no significant changes in LAD coronary vascular resistance during sympathetic stimulation. During 75 mM potassium infusion, however, the increase in LAD flow was significantly less than control at 5 and 20 Hz ansa stimulation (Figure 6.4a and Table 6.1) with a corresponding increase in coronary vascular resistance at these stimulation frequencies (Figure 6.4b and Table 6.1). Mean circumflex coronary flow and coronary vascular resistance were not significantly altered by the infusions. As noted in the preliminary experiments, increases in flow tended to be greater in the circumflex than the LAD territory.

Self terminating ventricular tachycardia was noted on two occasions during high frequency ansa stimulation with 75 mM potassium. Ventricular fibrillation did not occur. In all

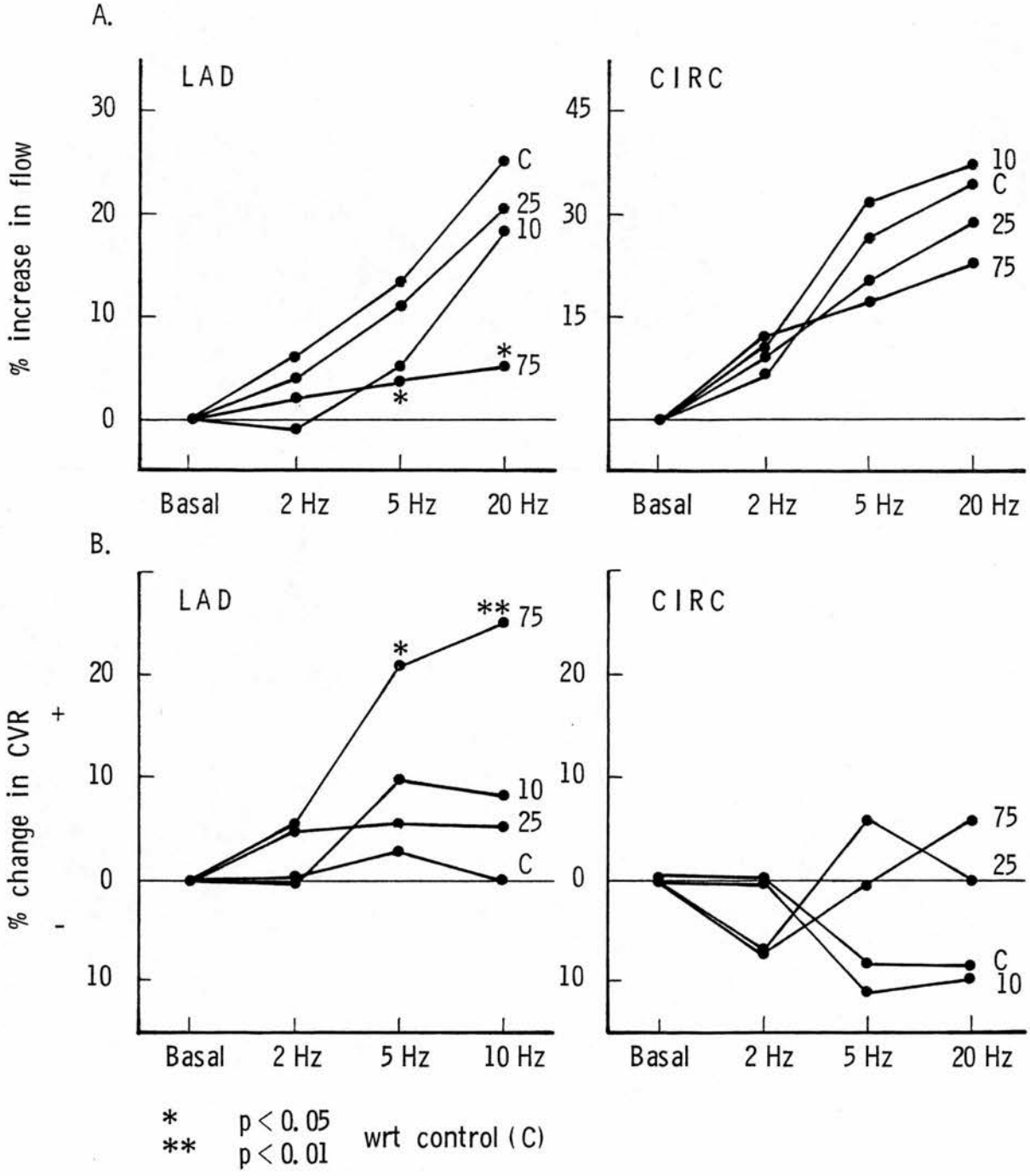


Figure 6.4 Mean haemodynamic responses to graded ansa stimulation (2, 5, 20 Hz). Effects of regional potassium infusion (10, 25, 75 mM) into LAD on flow (A) and coronary vascular resistance (CVR; B) in LAD and circumflex (CIRC) territories (n=6).

	2 Hz		5 Hz		20 Hz	
	Flow	CVR	Flow	CVR	Flow	CVR
CONTROL						
LAD $\bar{x}$	7	-1	13	3	26	0
SEM	3	6	5	7	11	8
CIRC $\bar{x}$	8	-1	25	-8	35	-8
SEM	3	5	8	7	14	8
75 mM KCl						
LAD $\bar{x}$	2	5	3	21	5	25
SEM	1	6	5	11	6	10
CIRC $\bar{x}$	7	-7	18	-1	23	6
SEM	3	5	9	10	12	10

Table 6.1: Changes in regional mean coronary blood flow (flow) and coronary vascular resistance (CVR) during graded ansa stimulation. Effects of high dose KCl into LAD. Results are expressed as per cent change from basal (pre-stimulation) values.

experiments, the surface PR interval remained constant, although in both experiments with ventricular tachycardia, some slurring of the ventricular electrogram was observed, suggesting delayed ventricular activation.

Lactate extraction was maintained across the heart throughout with the exception of high frequency ansa stimulation during the 75 mM potassium infusion when lactate release from the myocardium averaged 60 $\pm$ 52 per cent ($0.01 > p > 0.05$ wrt basal A-V difference). Arterial lactate increased slightly during the infusions from 0.8 $\pm$ 0.3 mmol/l during the initial to 1.3 $\pm$ 0.5 mmol/l during the final saline infusion ($0.1 > p > 0.05$).

Series II: Adenosine

Prior to sympathetic stimulation, intracoronary adenosine at the two highest doses resulted in significant spontaneous release of small amounts of [NA] from the LAD territory (-0.1 $\pm$ 0.4 pmol/ml control; 0.6 $\pm$ 0.7 pmol/ml 10^{-3}M adenosine; 1.0 $\pm$ 0.6 pmol/ml 10^{-2} M adenosine; p for both < 0.05; Figure 6.5a). [NA] release at 2 and 5 Hz ansa stimulation was similar for all doses of adenosine. At 20 Hz, however, release was inhibited at the two highest doses (3.8 $\pm$ 1.4 pmol/ml control; 0.4 $\pm$ 0.7 pmol/ml 10^{-3} M adenosine; 0.7 $\pm$ 0.6 pmol/ml 10^{-2} M adenosine; p for both $<$ 0.05; Figure 6.5a). Thus at these two infusion rates, [NA] release was independent of ansa stimulation. No significant differences in [NA] release from the lateral circumflex territory were observed during adenosine infusion (Figure 6.5b).

As expected, adenosine caused progressive coronary

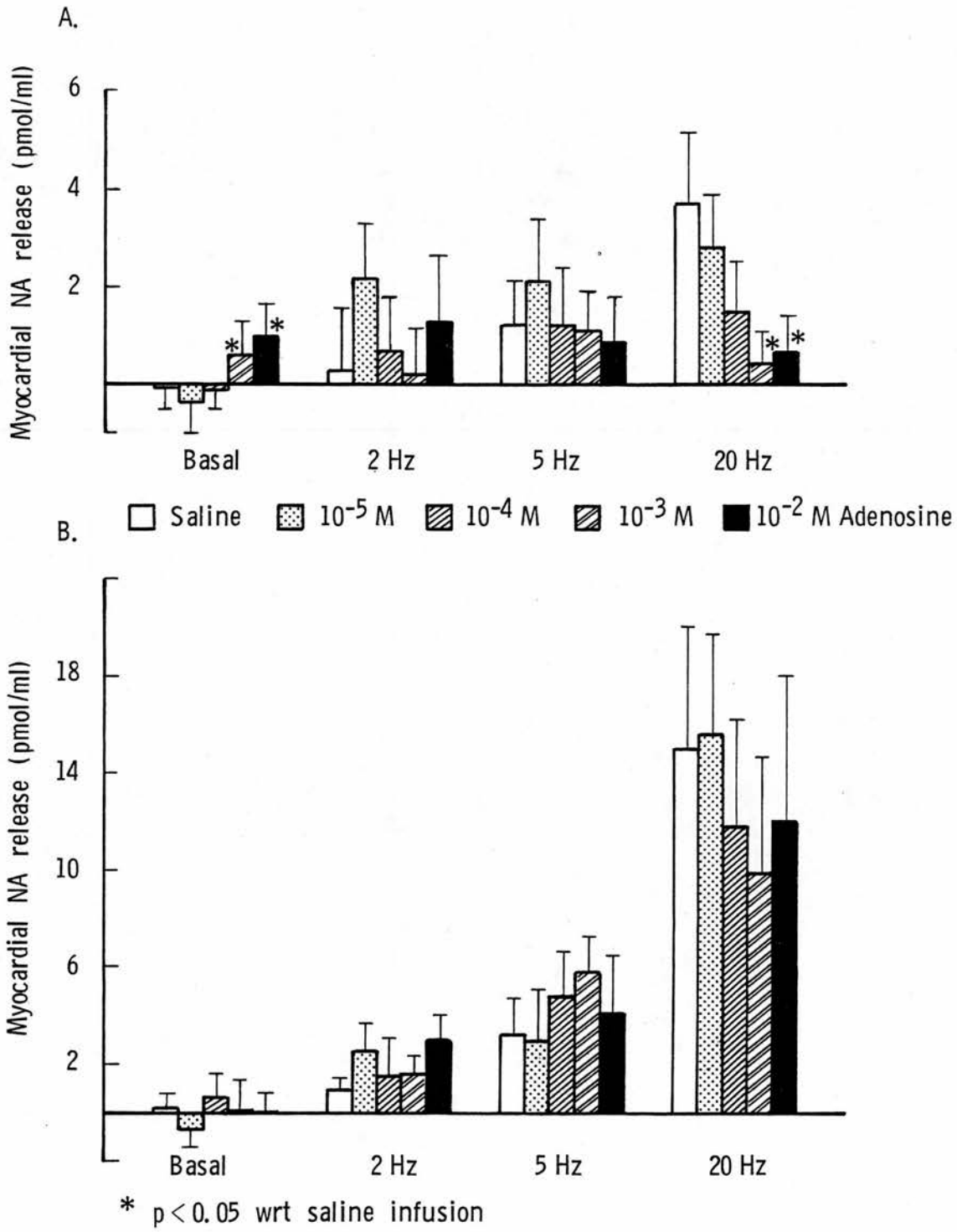


Figure 6.5 Myocardial NA release from anterior (A) and lateral (B) veins during graded ansa stimulation; effects of regional adenosine infusion (n=5; mean ± SEM) into LAD territory.

vasodilation with a rise in basal LAD flow from 29 ± 3 ml/min during the saline infusion to 87 ± 13 ml/min during the highest dose of adenosine (Table 6.2), corresponding with an approximately four-fold reduction in vascular resistance. Blood pressure was unchanged at all except the highest dose of adenosine which caused a small fall (Table 6.2). Coronary vascular resistance in the circumflex territory remained unchanged.

Changes in LAD and circumflex blood flow (Figure 6.6a) and coronary vascular resistance (Figure 6.6b) during ansa stimulation were not significantly modified by adenosine, despite major alteration in coronary vascular tone.

Lactate extraction was maintained across both areas of myocardium during intracoronary infusions and ansa stimulation and averaged 26 ± 11 per cent. Arterial levels remained constant during the infusions (0.9 ± 0.2 mmol/l during the initial and 1.1 ± 0.4 mmol/l during the final saline infusion).

Discussion

The main finding of this investigation is the demonstration that both potassium and adenosine, metabolites that accumulate in ischaemic myocardium, can modify local sympathetic nerve terminal activity, as measured by regional myocardial [NA] release. Thus the local control of myocardial catecholamine release in acute ischaemia may involve both 'adrenergic' and 'metabolic' feedback.

Extracellular accumulation of potassium in ischaemic dog myocardium proceeds rapidly during the first five minutes after coronary ligation and thereafter rises more slowly (Benzing et al,

		ADENOSINE				
		Control	10^{-5}M	10^{-4}M	10^{-3}M	10^{-2}M
Basal flow (ml/min)						
LAD	$\bar{x}$	29	31	39	70	87
	SEM	3	3	5	11	13
CIRC	$\bar{x}$	56	57	54	58	60
	SEM	8	10	8	10	9
Aortic Diastolic Pressure (mmHg)						
	$\bar{x}$	96	96	95	94	82
	SEM	3	4	5	3	7
CVR (units)						
LAD	$\bar{x}$	3.3	3.1	2.4	1.3	0.9
	SEM	0.2	0.3	0.4	0.3	0.2
CIRC	$\bar{x}$	1.7	1.7	1.8	1.6	1.4
	SEM	0.2	0.2	0.2	0.2	0.2

Table 6.2: Regional myocardial blood flow, aortic diastolic pressure and coronary vascular resistance during adenosine infusion to LAD.

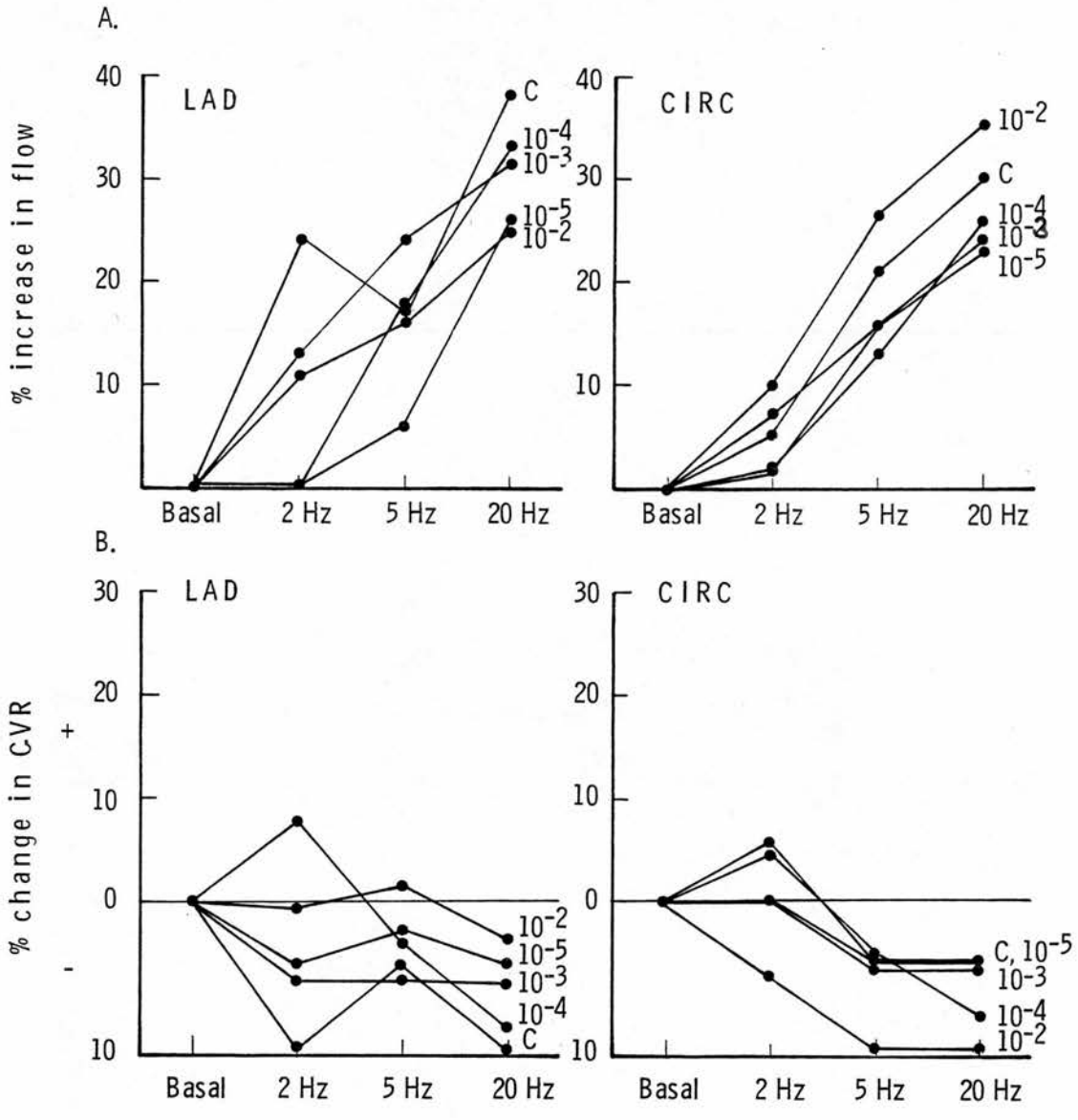


Figure 6.6 Mean haemodynamic responses to graded ansa stimulation (2, 5, 20 Hz). Effects of regional adenosine infusion (10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}M) into LAD on flow (A) and coronary vascular resistance (CVR:B) in left anterior descending (LAD) and circumflex (CIRC) territories (n=5).

1972; Wiegand et al, 1979). Early studies evaluating the effects of regional hyperkalaemia on conduction delay and arrhythmias reproduced many of the electrophysiological abnormalities seen during acute ischaemia, including ventricular fibrillation (Logic 1972, 1973). Furthermore, non-uniform distribution of potassium increments within epicardial and endocardial layers was observed (Ettinger et al, 1973), similar to the heterogeneous distribution of ischaemic metabolites during coronary occlusion (Russell, 1982). It is likely, however, that the concentrations of infused potassium in these studies were greater than the extracellular levels found during acute ischaemia especially in the dog model.

Thus, the infusion rates and concentrations selected in this study attempted to mirror the concentration ranges likely to occur across an ischaemic area in the dog model. Plasma levels in the local venous drainage of the perfused territory increased by 0.1, 1.3 and 6.1 mM respectively at the three concentrations, certainly an underestimate of the extracellular concentration, although this was not measured. Potassium levels in the unperfused territory were unchanged.

At these infusion rates, a biphasic action of regional hyperkalaemia on nerve stimulated [NA] release was observed; inhibition of release at the lowest concentration with potentiation of release at the highest concentration. No significant effects of potassium on [NA] release were observed before or during low frequency sympathetic stimulation (although the trend was similar) possibly because of relatively small changes in [NA] at these times. By contrast, [NA] release from the lateral circumflex territory remained unchanged throughout the periods of

hyperkalaemia, so that variability in effectiveness of sympathetic stimulation or deterioration in the preparation could not have explained the results.

The ability of regional hyperkalaemia to have disparate effects on sympathetic nerve terminal [NA] release in vivo illustrates the complexities of predicting the likely responses during acute ischaemia. However, it may be readily envisaged that areas of enhanced [NA] release resulting from potassium induced nerve terminal depolarisation or inhibition of neuronal reuptake (Shore 1972) adjacent to areas of inhibited [NA] release (from milder hyperkalaemia) will intensify the heterogeneous effects of the hyperkalaemia on regional activation times and repolarisation and increase the likelihood of arrhythmias. An increase in [NA] release may itself increase extracellular potassium accumulation and initiate a cycle of catecholamine release and electrophysiological abnormality. Enhanced beta-adrenoceptor stimulation of myocytes in a milieu of high extracellular potassium is conducive to re-entrant arrhythmias, possibly by facilitating slow response action potentials (Wit and Bigger, 1975). Beta-adrenoceptor blockade attenuates the initial rise in extracellular potassium independent of changes in heart rate (Wiegand et al, 1979). Thus, the possibility of combined effects of hyperkalaemia and myocardial [NA] release as triggers of early ventricular arrhythmias has both theoretical basis and experimental support (Hirsche et al, 1982). The changes in [NA] release shown in this study are analogous to in vitro studies in a variety of preparations (Haddy 1975; Verhaeghe et al, 1978).

The second interaction between changes in extracellular

potassium and neurosympathetic responsiveness centres round changes in coronary vascular resistance. At the highest infusion rate, sympathetic stimulation at 5 and 20 Hz resulted in significant increases in coronary vascular resistance and attenuated the rise in regional flow (Figure 6.4). Basal coronary flow was not affected, thus it is unlikely that the vasoconstriction occurred as a direct effect of hyperkalaemia on coronary vasculature. Indeed, in most vascular beds an increase in potassium causes local vasodilation (Kjellmer 1965; Haddy et al, 1968; Mellender and Johannson, 1968).

It is more likely that the vasoconstrictor response during sympathetic stimulation represents an interaction between hyperkalaemia and the responsiveness of the coronary vasculature to released [NA]. Vasoconstriction during sympathetic stimulation has been shown at several points within the range of coronary vascular reserve, including reactive hyperaemia (Schwartz and Stone, 1977) exercise (Murray and Vatner, 1979), adenosine infusion (Johannson et al, 1982) and in the presence of a coronary stenosis (Buffington and Feigl, 1981). Recently, Heusch and Deussen (1983) showed a progressive change in the effect of left cardiac sympathetic nerve stimulation on the resistance of the coronary vascular bed distal to an increasingly severe coronary stenosis. Sympathetic stimulation decreased the diastolic coronary resistance of intact coronary arteries but increased the resistance in the presence of a severe stenosis, resulting in lactate production and spontaneous ventricular fibrillation. The increase was blocked by post junctional alpha 2-adrenoceptor blockade. Although the mechanism of the increase in coronary vascular resistance during hyperkalaemia is speculative, enhanced alpha-adrenoceptor mediated

vasoconstriction seems possible, although impaired beta-adrenoceptor mediated or metabolic vasodilatation is not excluded. The potassium-induced increase in coronary vascular resistance during sympathetic activation provides one mechanism to explain vasoconstriction in the presence of myocardial ischaemia, although it remains contraversial whether such an effect could overwhelm vasodilation from accumulation of powerful vasodilator metabolites (Mudge et al, 1979; Gewirtz et al, 1982).

Tissue adenosine increases rapidly during acute myocardial ischaemia, with a seven fold increase in one study after only 15 seconds coronary occlusion (Olsson, 1978). Sympathetic stimulation further increases adenosine production which closely parallels changes in myocardial oxygen demand (Miller et al, 1979). The concentration of adenosine in the interstitial space during myocardial ischaemia has not been accurately determined, partly because of uncertainty as to whether adenosine formation in the heart is sarcolemmal (based on the activity of 5' nucleotidase) and hence close to the interstitial space (Rubio et al, 1977) or whether it is formed intracellularly by a cytoplasmic enzyme (5-adenosyl homocysteine hydrolase) and transported across the sarcolemmal membrane (Schutz et al, 1981). Nonetheless, concentrations of between 10^{-6} and 10^{-5} have been suggested in the interstitial space. Despite the rapid inactivation of adenosine, (Fredhold and Hedqvist, 1978), this should have been achieved by the higher infusion concentrations in this study.

The effects of intracoronary adenosine on myocardial [NA] release were different to those of potassium. Under basal conditions, high dose adenosine (10^{-3} and 10^{-2} M) increased [NA]

release selectively from the infused territory (Figure 6.5). The mechanism of this enhanced release is uncertain, since washout of released transmitter would also be enhanced from the increase in flow, thus reducing exposure to neuronal and extraneuronal uptake and metabolism. Adenosine-induced coronary vasodilatation does not appear to increase arteriovenous shunting in the coronary circulation (Downey et al, 1979). However, enhanced neuronal leakage of [NA] from storage vesicles in the nerve terminal is an alternative explanation for which there is experimental support from in vitro studies (Verhaeghe et al, 1977) and indirect support from the observation that adenosine releases cAMP into coronary venous blood (Huynh-Thu and Lammerant, 1980).

During sympathetic stimulation, however, myocardial [NA] release was inhibited during high frequency ansa stimulation at the two highest concentrations of adenosine, analogous to the dose-dependent inhibition of neurotransmission shown in a variety of tissues in vitro (Enero and Saidman, 1977; Fredholm and Hedqvist, 1978; Hedqvist and Fredhold, 1979). This presynaptic action of adenosine will be complementary to post synaptic inhibition of the inotropic and chronotropic responses to catecholamines (Schrader et al, 1977, 1982), and may explain suppression of sinus node automaticity and prolongation of atrioventricular nodal conduction (Di Marco et al, 1983).

Despite inhibition of regional [NA] release during ansa stimulation, changes in coronary blood flow and coronary vascular resistance were unaffected at all adenosine concentrations. A trend for reduction in coronary vascular resistance was evident during sympathetic stimulation in both the LAD and circumflex

territories but this did not achieve statistical significance.

Thus, these investigations have shown that the profile of sympathetic nerve terminal activity in acutely ischaemic myocardium appears to be very complex, involving heterologous (metabolite) as well as homologous (catecholamine) regulation of activity. Only two potentially active metabolites were chosen for study although it is likely that many others (such as pH, adenine nucleotides, 5-hydroxytryptamine lactate etc.) may be active in determining [NA] release from different areas of ischaemic heart.

7 INFLUENCE OF BETA-ADRENOCEPTOR ANTAGONISM ON NERVE TERMINAL RESPONSIVENESS, BLOOD FLOW AND ACTIVATION ABNORMALITIES DURING ACUTE ISCHAEMIA: THE IMPORTANCE OF HEART RATE

A variety of beta-adrenoceptor antagonists have been evaluated and shown to be variably effective in the prevention of ventricular arrhythmias during experimental myocardial ischaemia and infarction (Corr and Gillis, 1978; Meesman 1982). However, controversy remains as to whether the differing pharmacological profiles of these drugs, namely selectivity, intrinsic sympathomimetic activity and membrane-stabilising activity are of relevance in any antiarrhythmic or anti-ischaemic action. The mechanism of action of beta-adrenoceptor antagonists in protecting ischaemic myocardium is incompletely understood and may involve presynaptic effects, antiplatelet effects and peripheral effects in addition to classical postsynaptic myocardial catecholamine antagonism. Some but not all of the metabolic actions of beta-adrenoceptor antagonists are dependent on haemodynamic changes (reduction in heart rate and blood pressure) after drug administration.

Several studies have suggested that intrinsic differences exist in the efficacy of beta-adrenoceptor antagonists in acutely ischaemic myocardium. Potassium release from the ischaemic region, for example, may play an important role in initiating ventricular arrhythmias (see Chapter 6). Catecholamines have biphasic effects on potassium release; propranolol and $beta_2$-selective antagonists block the beneficial decrease in potassium release (Lockwood and Lum, 1974). Practolol, an antagonist with both beta-selectivity and intrinsic sympathomimetic activity, has been shown to reduce the incidence of ventricular fibrillation after coronary artery occlusion (Sethi et al, 1973; Kelliher et al, 1975). Practolol, but not propranolol, increases the diastolic period for subendocardial perfusion and does not unmask alpha-adrenoceptor mediated coronary vasoconstriction in the ischaemic area (Marshall

and Parratt, 1976).

In view of the reported anti-arrhythmic actions of low, non-pressor doses of systemic catecholamines, associated with reduction in myocardial potassium loss (Regan et al, 1970), it was hypothesised that the use of a beta-adrenoceptor antagonist with intrinsic sympathomimetic activity might have the dual actions of preventing the detrimental effects of heterogeneous noradrenaline release within the ischaemic area while maintaining low level but uniform sympathetic tone.

Pindolol is a potent beta-adrenoceptor antagonist, which as a result of indole substitution of the aromatic ring possesses partial agonist activity (Kaufmann and Blinks, 1980). Compared to pure antagonists, the drug causes a relatively modest fall in heart rate, blood pressure and global left ventricular function in the anaesthetised dog model (Stephen et al, 1978) and in man (Svendsen et al, 1979).

This series of experiments has evaluated the effect of intravenous pindolol on sympathetic nerve terminal activity, regional myocardial blood flow distribution, arrhythmias and conduction abnormalities during LAD occlusion in the dog model previously described.

Methods

The experimental preparation was as described in Chapter 4. Briefly, in the open chest pentobarbitone anaesthetised dog, the heart was suspended in a pericardial cradle and sampling catheters placed in the aorta ,in a vein in the centre of the LAD territory

and laterally in a vein draining towards the coronary sinus within the circumflex territory. Regional anterior myocardial ischaemia was induced by 12 minute periods of LAD ligation using an occlusion clip. The anterior and posterior ansa from the left stellate ganglion were stimulated by square wave pulses (4 msecs) at supramaximal voltage (8-10v) and varying frequency (1-10 Hz), allowing assessment of minute-to-minute changes in myocardial catecholamine and lactate overflow from ischaemic and non-ischaemic myocardium during LAD ligation and reperfusion and during sympathetic stimulation.

Regional epicardial activation delay across the ischaemic area was mapped using a flexible 80 point multielectrode grid as described in Chapter 2.

Detailed mapping of regional myocardial blood flow was achieved by left atrial injection of large numbers of microspheres (at least 8 x 10^6) labelled either with ^{57}Co or ^{113}Sn. This allowed the heart to be sectioned into 80 full thickness tissue samples of the left ventricular wall (approximately 5 mm square) and each biopsy to be divided into endocardial and epicardial halves, while still retaining the minimum number of spheres in each biopsy for accurate assessment of regional myocardial blood flow (Buckberg et al, 1971). Previous studies have confirmed the reproducibility of flow determination with high dose microspheres labelled with these isotopes and the absence of haemodynamic effect of the injection itself (Russell et al, 1982).

Regional myocardial catecholamine and lactate release, epicardial activation delay and blood flow maps were performed during two twelve minute periods of LAD occlusion and reperfusion

as shown in Figure 7.1. Ansa stimulation was performed for 30 seconds at low (1 Hz) and high (10 Hz) frequency sequentially on two occasions 4 1/2 and 9 1/2 minutes after coronary occlusion. Regional myocardial flow was measured three minutes after coronary occlusion and activation delay assessed at three and nine minutes of ischaemia, one minute after coronary reperfusion, and immediately after each period of high frequency ansa stimulation (Figure 7.1). At least one hour recovery was allowed between successive occlusions.

Three experimental groups were evaluated.

(1) A control group (n = 8) without drug administration during either occlusion and with atrial pacing at constant rate throughout;

(2) A second group (n = 8) with a control occlusion followed by intravenous pindolol 0.45 mg/kg i.v. thirty minutes before the second occlusion, also with atrial pacing at constant rate throughout and

(3) A third group (n = 8) similar to the second group but without atrial pacing during either occlusion.

In addition the effects of three doses of pindolol on myocardial [NA] release during graded ansa stimulation in the absence of ischaemia were investigated (n = 3).

Dose-response curves (heart rate and pulse pressure) to bolus injections of isoprenaline (1, 2.5, 5, 10 μg pre and 10, 25, 75, 200 μg post pindolol) were determined in the unpaced group 15 minutes before each period of ischaemia.

Ventricular fibrillation was managed by internal defibrillation after removal of the coronary occlusion clip. All

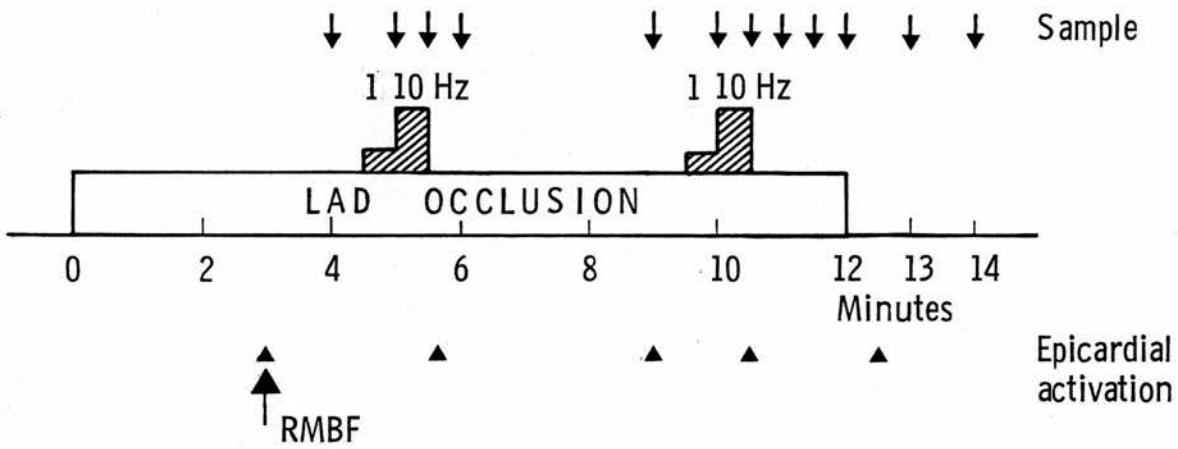


Figure 7.1 Experimental protocol. See text for details. Bars indicate ansa stimulation at low and high frequency.

samples obtained after the onset of ventricular fibrillation were excluded from analysis.

Changes in catecholamine and lactate concentrations for the three groups were evaluated by analysis of variance and computed modified t statistic. Intraexperimental comparisons (for pair differences) were validated by reproducible changes in catecholamine release, blood flow and electrophysiology during two successive occlusions in the control group. Non-parametric methods were used for analysis of changes in flow and surface electrophysiology, although variability in epicardial and endocardial flow has been expressed by the use of variance. Unless otherwise stated, data are expressed as mean $\pm$ SEM. A five per cent level of confidence was considered statistically significant.

Results

Catecholamines:

Basal [NA] release from the normal (non-ischaemic) heart was not modified by pindolol at any of the three doses selected (Figure 7.2). However, at the two higher doses (0.15 and 0.45 mg/kg), a small reduction in peak release was observed during maximal sympathetic stimulation. No inhibition of [NA] release was observed at lower stimulation frequencies, although peak concentrations in anterior and lateral coronary venous effluent were modest at these frequencies.

Mean changes in [NA] at each of the three sampling sites during LAD occlusion, ansa stimulation and coronary reperfusion are shown for the three groups in Figure 7.3. In the control group,

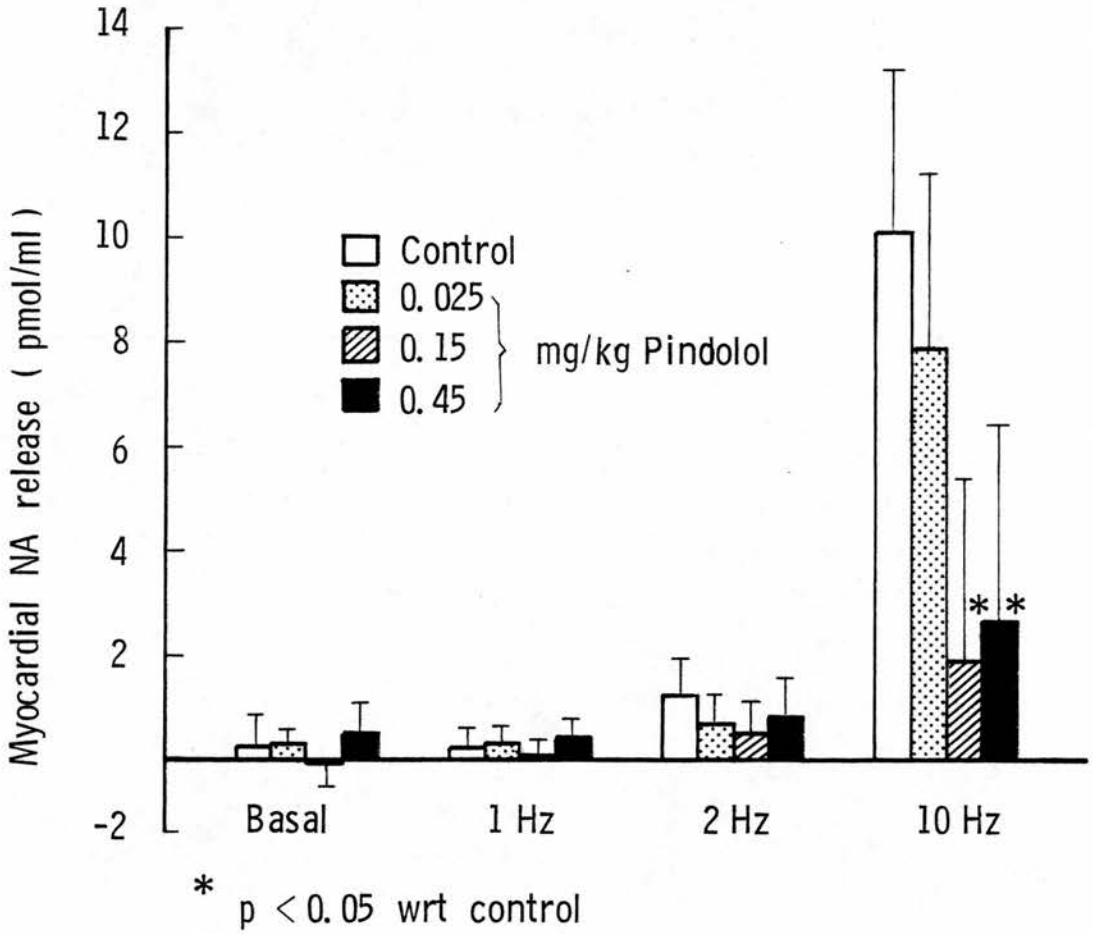


Figure 7. 2 Peak noradrenaline release (mean + SEM) during graded ansa stimulation for one minute. Effects of intravenous pindolol. Mean data for anterior and lateral coronary vein combined (n=6; 3 expts).

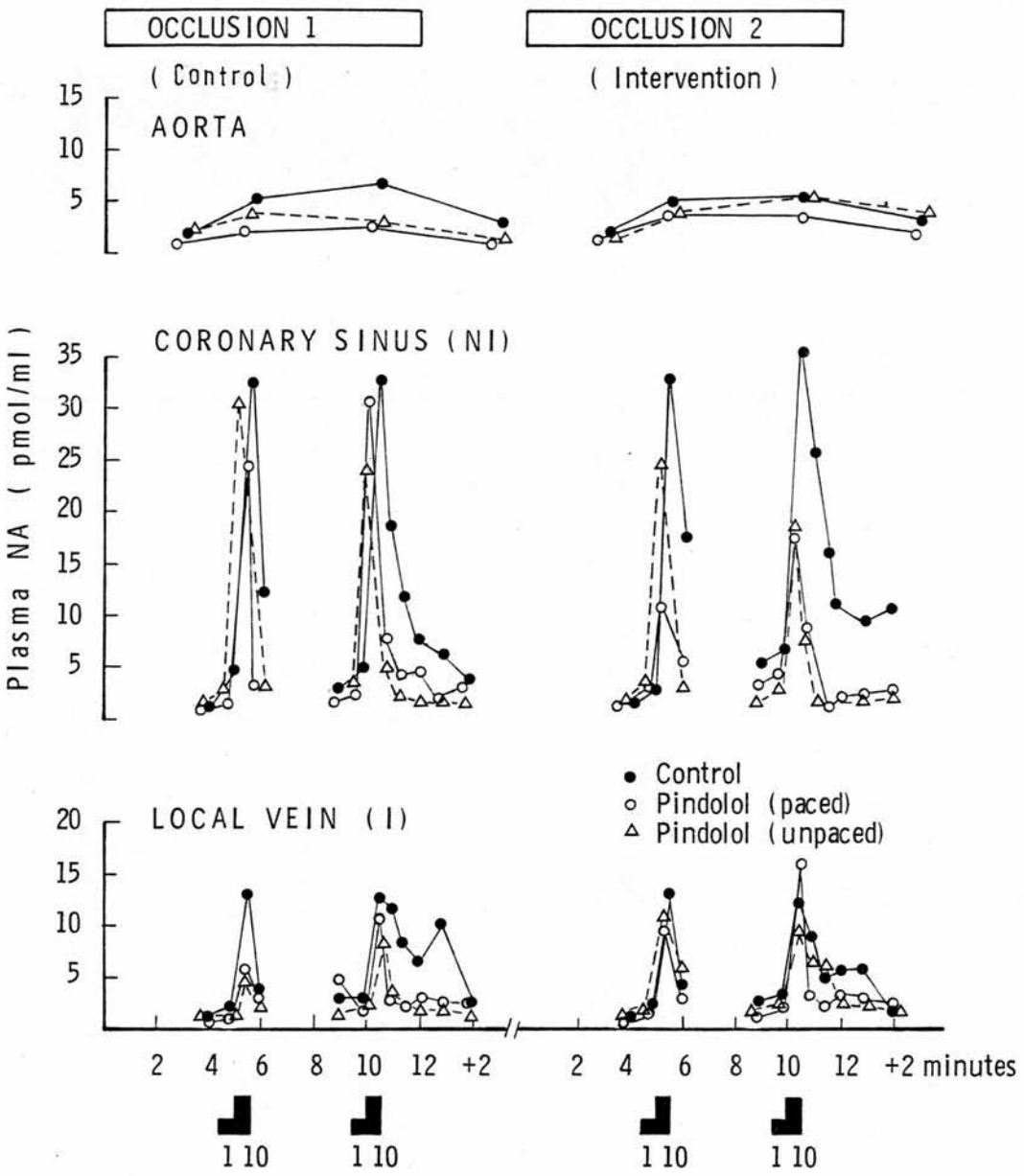


Figure 7.3 Mean plasma noradrenaline (NA) responses to ansa stimulation during LAD occlusion and on reperfusion (n=8 each group). Effects of pindolol. Blocks indicate low (1 Hz) and high (10 Hz) frequency ansa stimulation.

arterial [NA] and nerve-stimulated increases in venous [NA] at both sampling sites were similar during the two periods of coronary occlusion and sympathetic stimulation. Arterial [NA] did not change following pindolol (1.1 $\pm$ 0.4 pmol/ml before and 1.5 $\pm$ 0.7 pmol/ml after drug administration), and the trend for a gradual rise in arterial [NA] during the experimental period was unaltered. However, as noted in the earlier studies without coronary occlusion, pindolol reduced peak [NA] responses in the non-ischaemic venous effluent. No such trend was observed across the ischaemic area. Peak [NA] release from the ischaemic and non-ischaemic areas at low and high frequency ansa stimulation is shown in Figure 7.4. At 10 Hz, pindolol reduced peak [NA] release from the non-ischaemic area from 25.3 $\pm$ 5.4 to 11.0 $\pm$ 8.9 pmol/ml ($p < 0.02$) in the paced series and from 26.1 $\pm$ 6.1 to 16.9 $\pm$ 7.6 pmol/ml ($p < 0.05$) in the unpaced series. No statistically significant significances in myocardial [NA] release from the ischaemic area were noted in any of the other three experimental groups.

As shown in earlier studies, arterial [A] increased progressively during the experimental period (Figure 7.5), especially during coronary reperfusion. No significant differences between the first and second periods of coronary occlusion were observed in any series. Spontaneous release of [A] from the heart did not occur.

Lactate:

Myocardial lactate extraction across the non-ischaemic area and production from the ischaemic area was similar for both

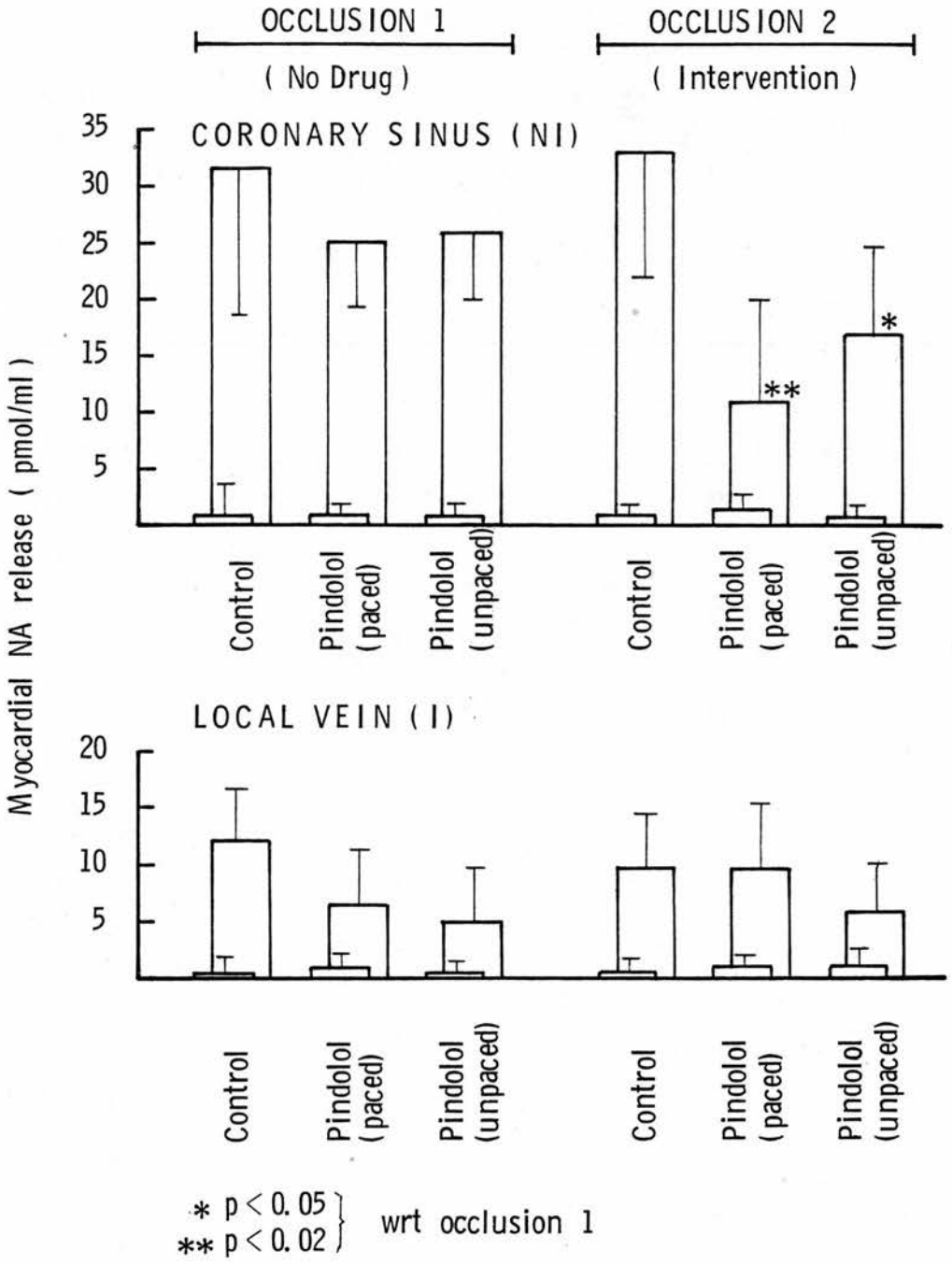


Figure 7.4 Peak myocardial NA release from ischaemic (I) and non-ischaemic (NI) areas during low (1Hz) and high (10Hz) frequency ansa stimulation. Effects of pindolol. Data shown (mean + SEM) for both periods of ansa stimulation.

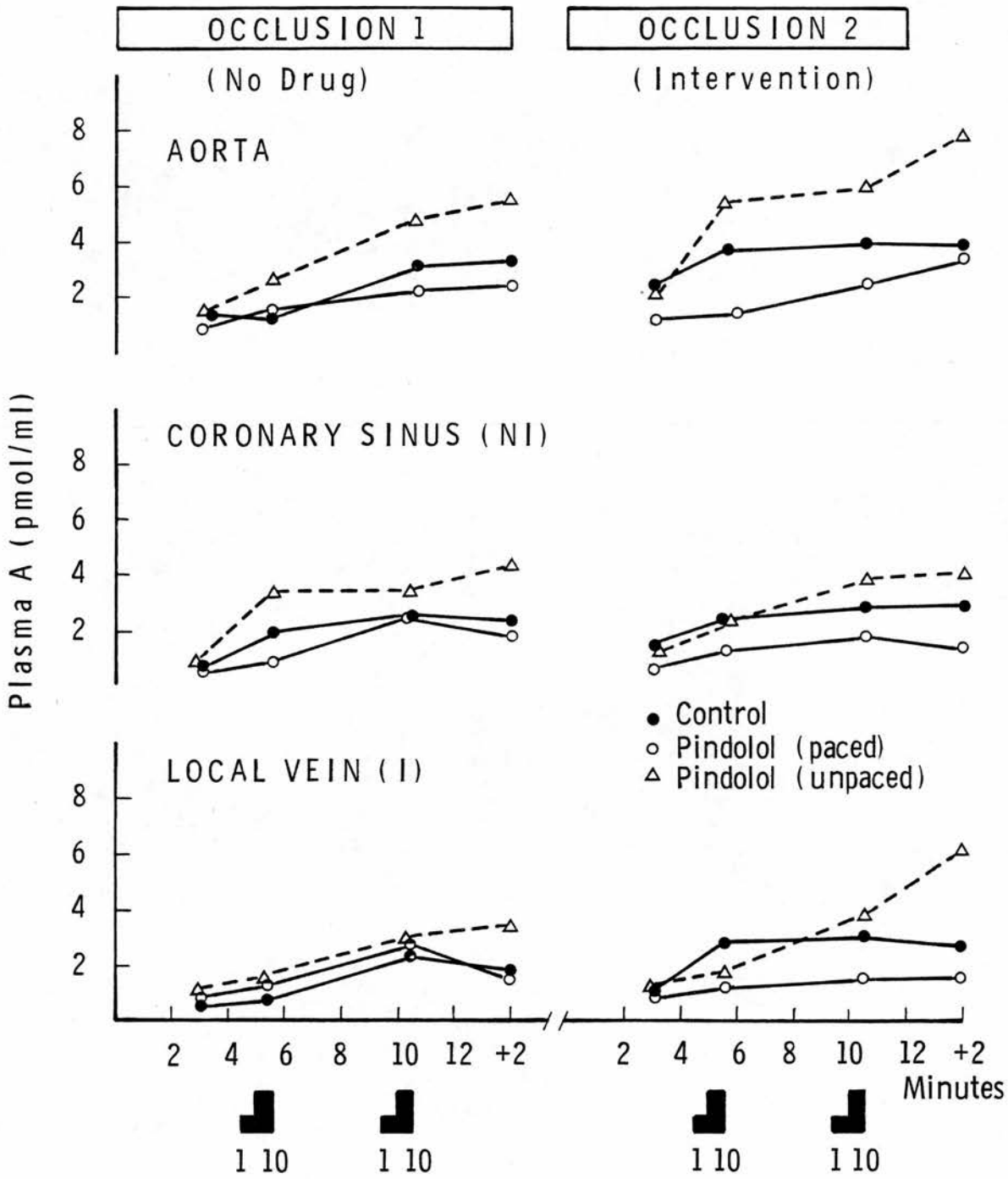


Figure 7.5 Mean plasma A responses during coronary occlusion and on reperfusion (n=8 each group). Effects of pindolol.

occlusions in the control series and in the pindolol series at constant heart rate (Figure 7.6). A significant reduction in myocardial lactate production (i.e. an increase in extraction) was however, seen during the second (pindolol) occlusion in the unpaced group. At three minutes, extraction after pindolol increased in this group from -133 ± 37 to -58 ± 32 per cent and during sympathetic stimulation it increased from -177 ± 45 to -77 ± 31 per cent (p for both < 0.05). Lactate extraction across the non-ischaemic area was unchanged. These changes are summarised in Figure 7.6.

Haemodynamics:

Heart rate for the control group averaged 185 ± 12 beats per minute and for the pindolol (paced) group 191 ± 13 beats per minute. The rate was kept constant throughout each experiment 5-10 beats per minute faster than the peak response during a test period of ansa stimulation. In the unpaced series, heart rate fell from 183 ± 8 beats per minute before to 151 ± 7 beats per minute after pindolol ($p < 0.001$). In keeping with the known acute effects of beta-adrenoceptor antagonism, mean blood pressure after pindolol fell from 103 ± 8 to 88 ± 8 mmHg in the paced series and from 97 ± 7 to 85 ± 7 in the unpaced series ($p < 0.05$). A small reduction in mean blood pressure in the control group between the first and second occlusions (from 101 ± 9 to 95 ± 6 mmHg) was not statistically significant.

The increase in mean systolic pressure during high frequency ansa stimulation was also attenuated by pindolol, although somewhat less in the paced compared to the unpaced group. The changes are

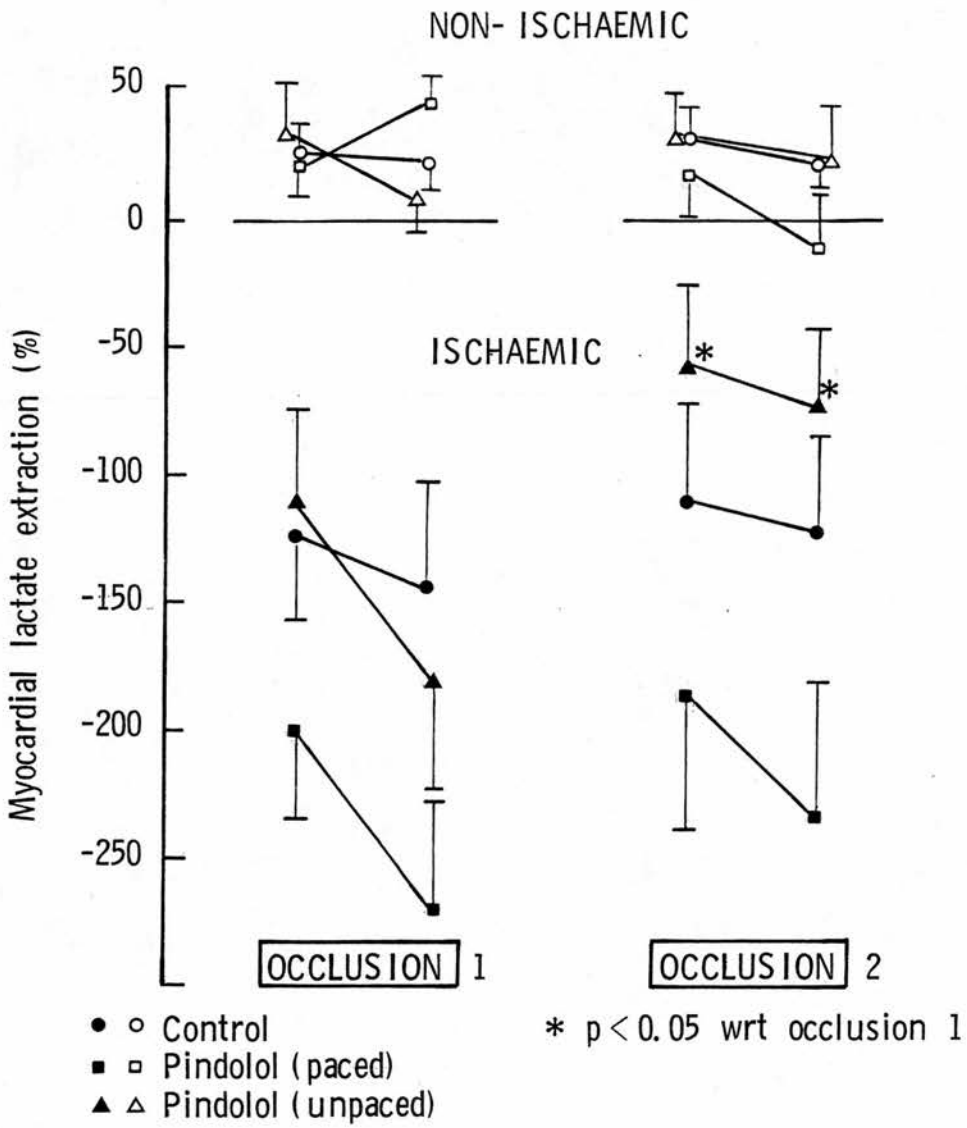


Figure 7.6 Myocardial lactate extraction (mean ± SEM) across non-ischaemic and ischaemic areas. Effects of pindolol. Extraction (art-ven/art &100) was measured firstly three minutes and secondly immediately after high frequency ansa stimulation (n=8, each group).

summarised in Table 7.1.

Isoprenaline dose-response curves for heart rate and pulse pressure before and after beta-adrenoceptor blockade are summarised in Figure 7.7. It can be seen that fairly complete beta-adrenoceptor antagonism has been achieved by this dose of pindolol, evidenced by an approximate 100 fold shift in the dose-response curves for these two variables.

Pindolol resulted in significant alterations in the pattern of and variability in blood flow within the central ischaemic and border ischaemic areas. Details of mean endocardial and epicardial flow in the non-ischaemic, border ischaemic and central ischaemic areas are shown in Figure 7.8. No differences in flow during the first and second occlusions were evident in the control group. Pindolol reduced flow to both ischaemic and non-ischaemic zones, although the reduction in the border and central ischaemic zone was relatively modest in the unpaced group, especially in the endocardial region.

Redistribution of flow within the central ischaemic area occurred with pindolol only in the unpaced group. Comparison between the endocardial:epicardial blood flow ratios in the paced and unpaced series is shown in Table 7.2. With pacing, the ratio fell slightly from 0.83 to 0.72 and from 0.77 to 0.59 in the border and central ischaemic areas respectively. With the reduction in heart rate in the unpaced group, the ratio increased slightly from 1.23 to 1.31 in the border ischaemic area and increased significantly from 0.72 to 0.93 in the central ischaemic area ($p < 0.05$).

	INCREASE IN MEAN PRESSURE			
	OCCLUSION 1 (No drug)		OCCLUSION 2 (Intervention)	
	1	2	1	2
CONTROL	23 ± 8	22 ± 7	24 ± 8	21 ± 8
PINDOLOL (Paced)	21 ± 5	19 ± 7	16 ± 6	16 ± 5
PINDOLOL (Unpaced)	29 ± 7	28 ± 7	16^{+} ± 9	12^{+} ± 9

$^{+}$ $p < 0.05$ wrt occlusion 1.

Table 7.1: Increments in mean blood pressure (mmHg) during two high frequency ansa stimulation periods: effects of pindolol.

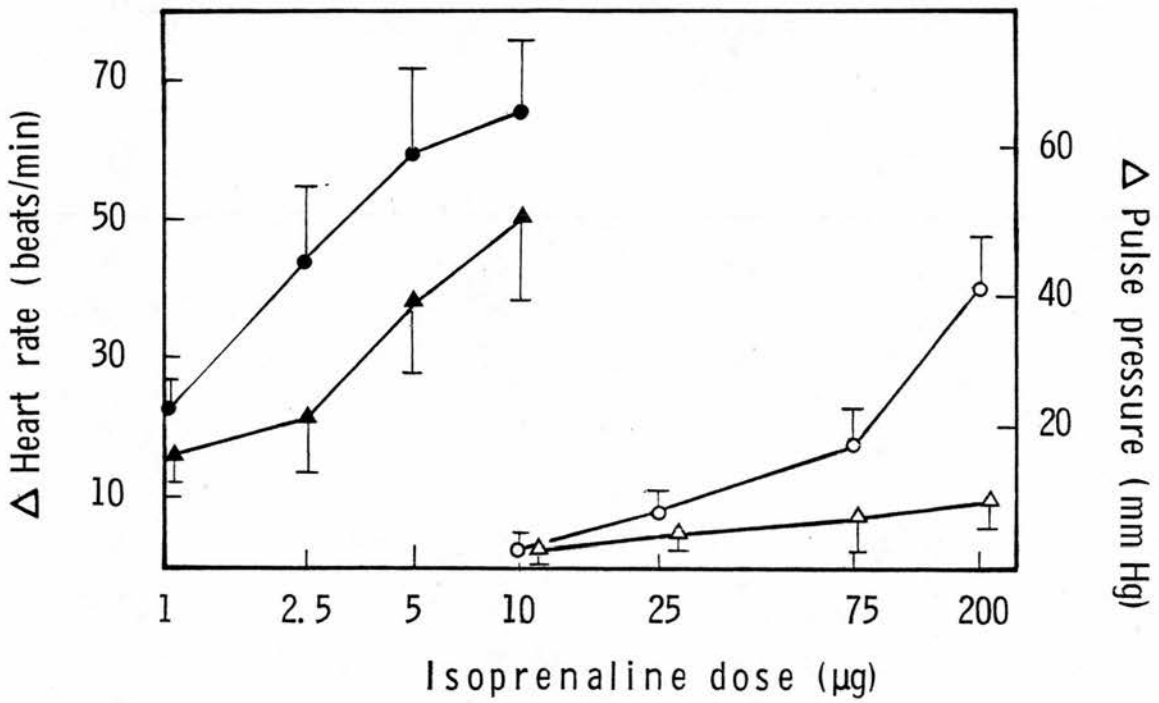


Figure 7.7 Dose-response curves (semi-log scale) for heart rate (circles) and pulse pressure (triangles) increments during bolus intravenous isoprenaline. Closed symbols before and open symbols after pindolol 0.45 mg/kg IV (unpaced series).

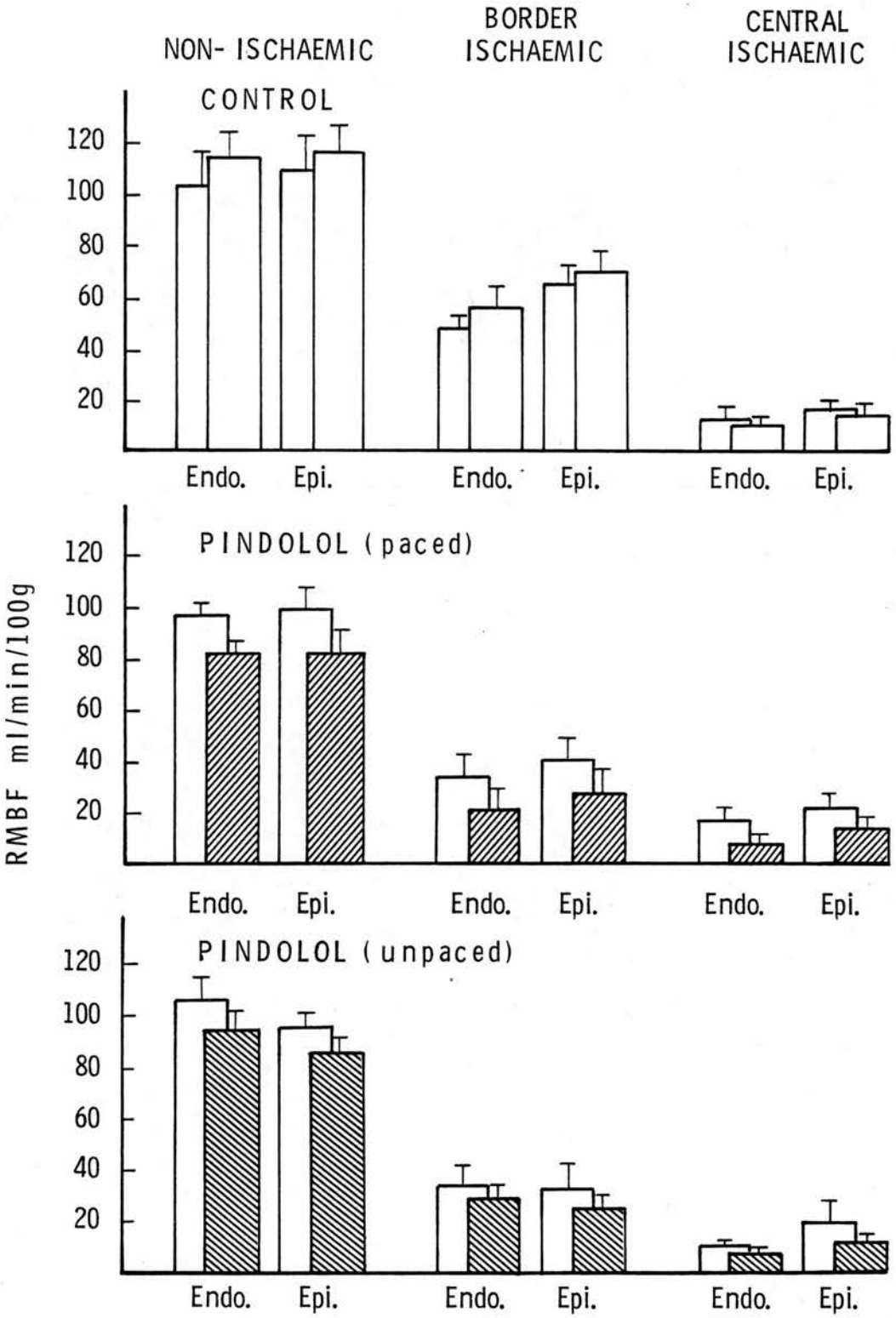


Figure 7.8 Regional myocardial blood flow (RMBF; mean ± SEM) to non-ischaemic, border ischaemic and central ischaemic areas. Effects of pindolol. Open histogram-no drug; hatched histogram-pindolol.

	PACED		UNPACED	
	CONTROL	PINDOLOL	CONTROL	PINDOLOL
NON ISCHAEMIC	0.98 ± 0.07	1.02 ± 0.10	1.12 ± 0.07	1.10 ± 0.09
BORDER ISCHAEMIC	0.83 ± 0.19	0.72 ± 0.23	1.23 ± 0.16	1.31 ± 0.32
CENTRAL ISCHAEMIC	0.77 ± 0.21	0.59 ± 0.19	0.72 ± 0.15	0.93+ ± 0.18

+ $p < 0.05$ Wrt control.

Table 7.2: Endocardial : epicardial blood flow ratios in the non-ischaemic, border ischaemic and central ischaemic areas. Effects of pindolol with and without atrial pacing.

Intraexperimental variability in regional blood flow was assessed by comparison of the variance of flow across each endocardial and epicardial region for each experiment. It can be seen from Table 7.3 that pindolol reduced flow variance across both the border and central ischaemic area, the reduction being somewhat greater in the unpaced compared to the paced series. No differences in flow variance were seen in the control series. Profiles of flow variability from one experiment from the unpaced series are shown in Figure 7.9. In addition to a reduction in flow, pindolol reduced the variability in regional flow, particularly in the ischaemic (low flow) region.

Electrophysiology:

Spontaneous ventricular fibrillation occurred four times in two control experiments. The time of onset of ventricular fibrillation was similar in these experiments, occurring respectively at 8 and 10 1/2 minutes after each LAD ligation in the first and 10 and 10 minutes after each LAD ligation in the second study. In the paced pindolol series, ventricular fibrillation developed three times during the control occlusion at 6, 10 and 10 1/2 minutes and twice immediately after coronary reperfusion. The pattern of arrhythmia development was similar after pindolol in this series, ventricular fibrillation occurring in the same three experiments during coronary occlusion (at 7, 5 and 10 minutes respectively) and three times after coronary reperfusion. In the unpaced pindolol series, however, spontaneous ventricular fibrillation developed three times during the control occlusion (at 4, 5 and 8 minutes) and once immediately after coronary reperfusion

	PACED			UNPACED		
	n	CONTROL	PINDOLOL	n	CONTROL	PINDOLOL
NON ISCHAEMIC	38 ± 6	1469 ± 349	1450 ± 342	24 ± 3	1117 ± 176	885 ± 165
BORDER ISCHAEMIC	34 ± 3	764 ± 211	576*± 210	19 ± 2	622 ± 102	398**± 64
CENTRAL ISCHAEMIC	31 ± 2	135 ± 26	91*± 28	20 ± 2	156 ± 37	85**± 24

* $P < 0.025$
** $P < 0.005$ wrt control

Table 7.3: Intraexperimental regional myocardial flow variance $(ml/min/100g)^2$. Effects of pindolol (paced and unpaced series). n = number of biopsies from which flow variance assessed. Data combined from endocardial and epicardial regions.

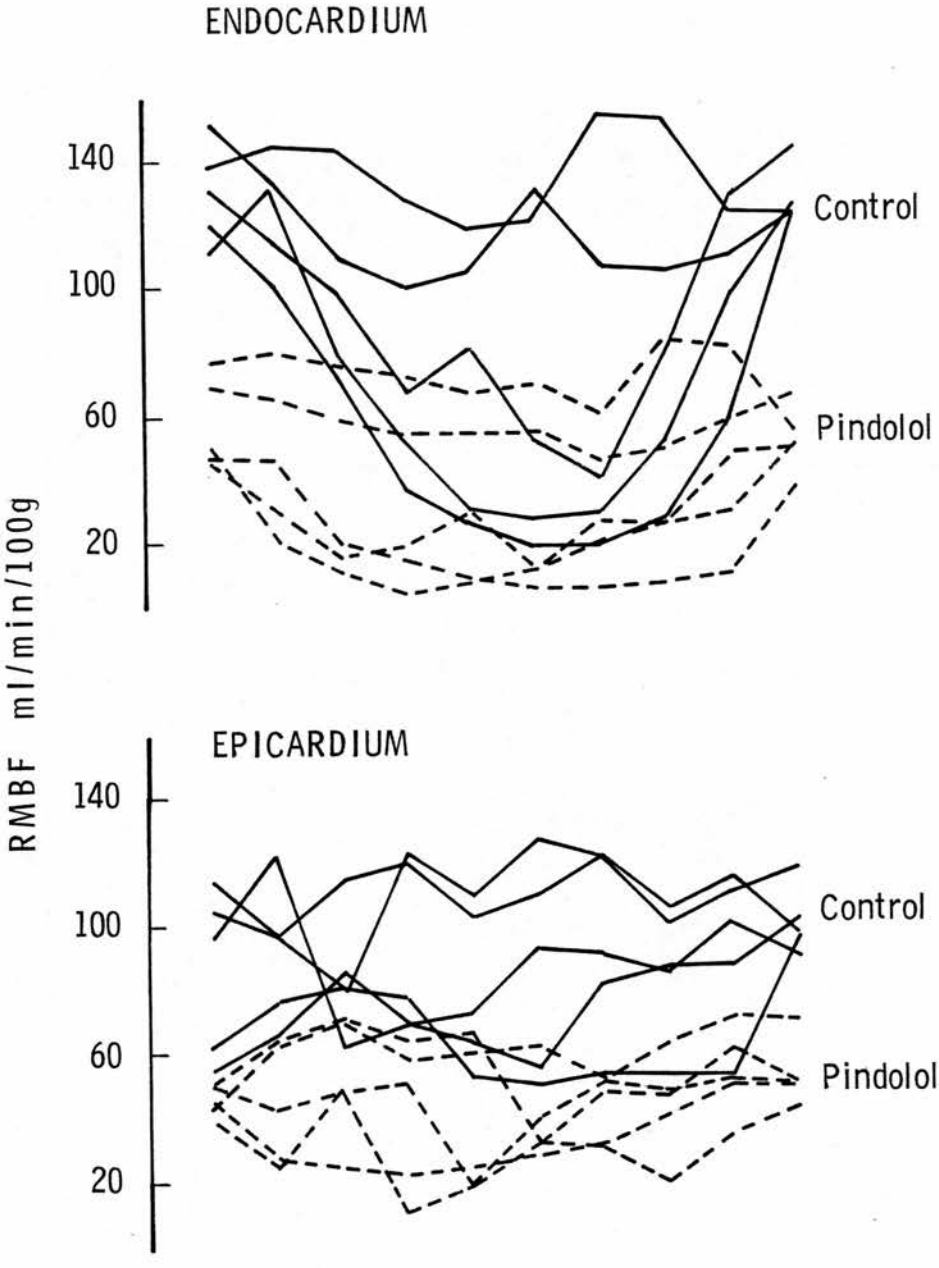


Figure 7.9 Patterns of flow variability from one experiment (unpaced series). Flow values in 50 individual biopsies from a 5 &10 grid across the ischaemic region.

but no episodes of ventricular fibrillation occurred during the second (pindolol) occlusion. Ventricular fibrillation on reperfusion developed after pindolol on three occasions, two of these in animals previously fibrillating during the ischaemic period. Thus an antiarrhythmic action of pindolol on ventricular fibrillation during acute ischaemia was evident only in the unpaced group.

Individual and mean epicardial activation delays during each period of coronary occlusion, sympathetic stimulation and reperfusion are summarised in Tables 7.4, 7.5 and 7.6 for the control, pindolol (paced) and pindolol (unpaced) series respectively. Mean activation delay within the ischaemic area was derived from those electrodes showing conduction delay equal to or greater than forty milliseconds at any time during coronary occlusion and sympathetic stimulation. The number of electrodes in each experiment (an index of the area of activation abnormality) is shown in the tables. No significant differences in activation delay in the first and second periods of ischaemia were seen in the control (Table 7.4) and in the paced pindolol (Table 7.5) series. Activation abnormalities were, in general, intensified during sympathetic stimulation and although the increase in mean activation delay following ansa stimulation was attenuated somewhat in the paced pindolol group compared to the corresponding control (Table 7.5), this was not statistically significant. By contrast, activation delay was significantly ($p < 0.05$) less during the second occlusion in the unpaced pindolol series three minutes after LAD occlusion and during both periods of ansa stimulation (Table 7.6). This attenuation of conduction abnormality during acute ischaemia paralleled the lower incidence of spontaneous ventricular

EXPT		ELECTRODES	EPICARDIAL ACTIVATION DELAY (msecs)				
			3min 10Hz		8min 10Hz		Reperfusion
(1)	C1	13	27	29	27	28	26
	2		29	28	28	29	26
(2)	C1	9	45	54	49	VF	—
	2		36	46	VF	—	—
(3)	C1	10	44	46	46	VF	—
	2		43	44	46	VF	—
(4)	C1	18	36	39	41	38	28
	2		37	40	46	41	26
(5)	C1	9	26	26	28	29	25
	2		27	28	29	31	25
(6)	C1	5	46	51	48	50	34
	2		50	55	50	55	37
(7)	C1	17	43	41	40	43	—
	2		37	38	43	39	—
(8)	C1	17	55	56	51	59	27
	2		47	53	55	56	29
Mean ± SEM							
	C1		40±3	43±4	39±6	41±6	28±2
	C2		38±4	42±4	42±4	42±5	29±2

Table 7.4: Epicardial activation delay during the first (C1) and second (C2) periods of LAD ligation and on reperfusion Control series. Mean values (± SEM) for paired data also shown with the number of electrodes within the ischaemic area (see text for definition).

EXPT		ELECTRODES	EPICARDIAL ACTIVATION DELAY (msecs)				
			3min	10Hz	8min	10Hz	Reperfusion
(9)	C	19	29	49	VF	--	--
	P		29	53	VF	--	--
(10)	C	34	32	35	38	49	VF
	P		33	34	44	46	VF
(11)	C	23	31	36	33	35	25
	P		29	29	31	32	24
(12)	C	20	33	53	36	VF	--
	P		47	VF	--	--	--
(13)	C	16	37	40	34	39	32
	P		35	38	38	38	VF
(14)	C	26	41	45	43	45	28
	P		38	40	41	42	28
(15)	C	24	42	48	47	VF	--
	P		41	44	VF	--	--
(16)	C	12	31	39	36	40	31
	P		34	36	37	39	31
Mean ± SEM							
	C		35±2	42±2	37±2	42±3	28±3
	P		36±2	39±3	38±2	39±2	28±4

Table 7.5: Epicardial activation delay during first (control) and second (Pindolol) LAD ligation. Paced series. Mean values (± SEM) for paired data also shown.

EXPT		ELECTRODES	EPICARDIAL ACTIVATION DELAY (msecs)				
			3min 10Hz		8min 10Hz		Reperfusion
(17)	C	51	41	70	64	69	VF
	P		35	54	58	65	VF
(18)	C	52	56	VF	--	--	--
	P		25	48	76	77	VF
(19)	C	39	61	VF	--	--	--
	P		44	58	49	44	VF
(20)	C	13	30	35	30	39	29
	P		30	32	32	33	28
(21)	C	9	24	29	27	30	24
	P		24	25	25	25	24
(22)	C	39	42	46	41	45	27
	P		37	42	44	41	24
(23)	C	21	36	49	39	53	35
	P		32	38	36	39	30
(24)	C	27	44	69	VF	--	--
	P		42	55	51	46	32
Mean ± SEM							
	C		42±4	50±8	40±7	47±7	29±2
	P		34^{+} ±3	41^{+} ±5	39±6	41^{+} ±6	27±2

$^{+}$ $p < 0.05$ wrt control

Table 7.6: Epicardial activation delay during first (control) and second (Pindolol) LAD ligation. Unpaced series. Mean values (± SEM) for paired data also shown.

fibrillaton in this unpaced group.

Detailed analysis of patterns of activation delay from one experiment from the unpaced and one from the paced pindolol series is presented in Figure 7.10. It can be seen from this figure that activation abnormalities were similar in the control and pindolol groups during pacing at the same heart rate (Figure 7.10a upper panel) but that activation abnormalities were more intense (i.e. activation times longer) in the control compared to pindolol groups without pacing (Figure 7.10a, lower panel). Profiles of epicardial activation delay during sympathetic stimulation showed similar variability in activation in the paced pindolol series (Figure 7.10b, upper panel) but much less variability in activation in the unpaced pindolol series (Figure 7.10b, lower panel).

DISCUSSION

As demonstrated in the control series, two sequential twelve minute periods of regional myocardial ischaemia were associated with reproducible patterns of sympathetic responsiveness, lactate release, regional myocardial blood flow, activation abnormalities and spontaneous arrhythmias. Thus, comparison of changes in these parameters as a result of intervention before the second period of ischaemia is valid and has the advantage of allowing paired data analysis thus avoiding inter-experimental variability.

The main finding of this investigation is the almost complete dependence of the beneficial effects of pindolol on electrophysiological abnormalities, blood flow distribution and lactate release on reduction in heart rate. In the absence of bradycardia, pindolol caused relatively few changes in these

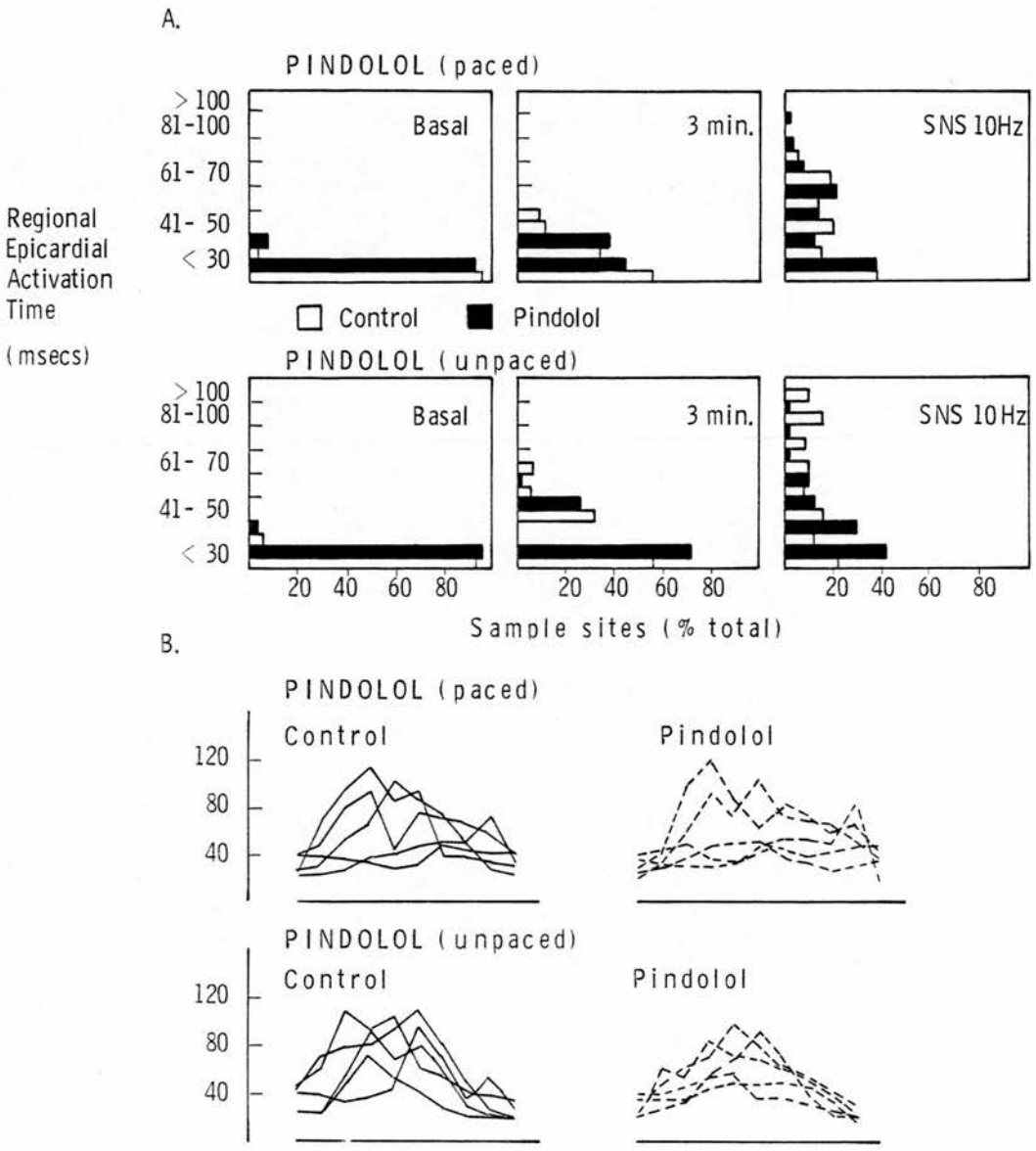


Figure 7.10 A Patterns of epicardial activation delay from one experiment in the paced and unpaced series. Activation delay is plotted against number of electrodes with that delay. Data are shown before and after pindolol prior to and three minutes after LAD ligation and during ansa stimulation. B Profiles of activation delay across the ischaemic zone from the same expt during ansa stimulation Data from 5 rows of 10 electrodes traversing the ischaemic area.

parameters. It is probable that bradycardia protects the acutely ischaemic myocardium by reduction in myocardial oxygen demand and hence acts as an ' anti-ischaemic' rather than an 'anti-arrhythmic' intervention.

Reduction in flow to the non-ischaemic area following beta-adrenoceptor blockade has been shown for virtually all beta-adrenoceptor antagonists (Becker et al, 1971, 1975; Gross and Winbury, 1973; Berdeaux et al, 1978). Redistribution of flow within the ischaemic region with an increase in the endo-epi flow ratio has been shown previously for several antagonists including pindolol (Flameng et al, 1975; Berdeaux et al, 1978) and has been variably attributed to combined $beta_1$- and $beta_2$-adrenoceptor blockade, unmasking of alpha-adrenoceptor mediated vasoconstriction in the epicardial zone and prolongation of diastolic perfusion time. The results of the present study suggest that it is the latter action, that predominates in this effect as the increase in the flow ratio was prevented by maintenance of a constant heart rate. In this respect the results are in agreement with the study by Gross and Winberg (1973) who noted that increasing the heart rate to control levels abolished the increase in endo-epi flow ratio following propranolol, practolol and bunolol. Since the major portion of subendocardial flow occurs during diastole, and since subendocardial flow is nearly always less than epicardial flow within the ischaemic area, it might be expected that prolongation of diastolic perfusion would enhance subendocardial perfusion. In a study by Warltier et al (1976) in the isolated canine heart, increases in heart rate alone were sufficient to decrease the endo-epi flow ratio, an observation supported by data from Neill et al (1973, 1975) in unaesthetised dogs and those with

a proximal coronary stenosis and from Flannery et al (1975a) during coronary vasodilatation. Although the effect of bradycardia alone was not studied, it is, however, unlikely that the increase in flow ratio with pindolol could be exclusively attributed to the fall in rate since Gross and Winberg showed that reduction in heart rate (SA node crush) within the range seen in the study presented here did not significantly modify the endo-epi ratio, a difference possibly explained by the different effects of these two manoeuvres on diastolic pressure - time index per minute.

A second action of pindolol on patterns of blood flow within the ischaemic territory was the reduction in flow variance within the border and central ischaemic areas. Measurement of variance of flow provides an index of the heterogeneity of flow distribution. Significant reductions in flow variance were seen after pindolol in both the paced and unpaced groups, although the reduction was more marked in the latter. Thus, beta-adrenoceptor blockade with this drug may reduce gradients of flow within the ischaemic area, an effect likely to promote more uniform changes in myocardial metabolism and possibly in electrophysiological disturbance. The combination of sympathetic stimulation and tachycardia is most likely to promote non-homogenous flow distribution in the heart (Flannery et al, 1975). However, absence of reduction in epicardial activation delay or spontaneous arrhythmias in the paced group suggests that this phenomenon may not be of primary importance in determining the electrophysiological abnormality.

The reduction in activation delay in the unpaced group after pindolol was reflected in a reduced incidence of spontaneous arrhythmias. No protective effects were observed in the paced

group during coronary occlusion or in either group during reperfusion. Several studies have shown that heart rate is a major determinant of myocardial electrical stability during ischaemia (Scherlag et al, 1970; Redwood et al, 1972; Kent et al, 1973). The protective effect of pindolol in this study is most likely to operate through this mechanism.

Reduction in lactate production across the ischaemic area with pindolol was also restricted to the unpaced group. Arteriovenous lactate gradients provide a fairly crude index of the extent of anaerobic myocardial metabolism and do not necessarily equate with the severity of ischaemic injury. During severe ischaemia, for example, lactate production may decrease because of acidaemia (Gevers,1977) but the venous concentration may be maintained because of a reduction in flow. Furthermore, the assumption that arteriovenous lactate differences equate with tissue concentrations can lead to important errors (Al Makdessi et al, 1982). However, in view of concomitant reduction in heart rate, spontaneous arrhythmias, conduction abnormalities and improvements in blood flow distribution, it seems probable that the reduction in lactate production in this group is a reflection of less severe derangement of myocardial metabolism, especially as the difference was evident by three minutes of ischaemia.

Reduction of peak [NA] release from the heart by pindolol during high frequency ansa stimulation supports the existance of presynpatic facilitatory beta-adrenoceptors in dog myocardium. The subclassification of these receptors has not been conclusively determined. Stjarne and Brundin (1975) and Westfall et al (1979) have suggested they are of the $beta_2$-subtype on the basis of the

action of selective agonists and antagonists, but Majewski et al (1980, 1981) have shown that metoprolol readily blocks the facilitatory action of adrenaline on catecholamine efflux from rat and guinea pig atria. A qualitatively similar reduction in [NA] release from dog heart was noted by Yamaguchi et al (1977) who studied the effects of sotalol on [NA] release during right cardio-accelerator nerve stimulation. Inhibition of release was greatest at lower stimulation frequencies, although marked increases in heart rate during high frequency stimulation during control studies may have influenced this result. The identity of the facilitatory transmitter during sympathetic stimulation is unknown but in vitro studies suggest that adrenaline is a more likely candidate that [NA] (Marjewski et al, 1981) as the former is much more effective at increasing [NA] release and can do so at a physiological concentration range (below 10 nM), close to that measured in arterial plasma in the pindolol experiments. It is also known that adrenaline may be taken up into sympathetic nerve terminals and released during sympathetic stimulation (Iversen and Whitby, 1962; Anden, 1964).

Although reduction in [NA] release during sympathetic stimulation may have theoretical benefits for the heart during regional ischaemia, a reduction in epicardial activation delay after pindolol was seen only in the unpaced group. Thus, at constant heart rate, no beneficial action was evident despite similar reduction in [NA] release. It may be important, however, that basal release of [NA] and release at lower (physiological) stimulation frequencies was not reduced by pindolol and that responses across the ischaemic area were unaffected at all stimulation frequencies.

8 QUANTITATIVE ANALYSIS OF MYOCARDIAL ADRENOCEPTORS - PROSPECTS AND LIMITATIONS

The classical pharmacological approach to the definition of receptor taxonomy, initiated by Dale in 1906, has been to compare rank order potencies of agonists and antagonists for a variety of physiological activities. Subtypes of beta-adrenoceptors were first suggested by Lands in 1967 but receptors remained a physiological concept until the mid 1970's when high specific activity radioligands were introduced permitting direct study of receptor binding sites in a variety of membrane fractions. Since methodology for studying beta-adrenoceptors was developed in 1974 (Aurbach et al, 174; Lefkowitz et al, 1974; Levitzki et al, 1974) and applied to the canine heart in 1975 (Alexander et al, 1975), there has been an explosion of information concerning molecular mechanisms of agonist-receptor interactions (Watanabe et al, 1982) and receptor modulation by physiological and pathophysiological processes (Lefkowitz, 1979; Matulsky and Insel, 1982).

Receptor modulation may provide an important mechanism for altered adrenergic responsiveness of the heart in disease and may result from homologous or heterologous interactions. Recognised homologous modulatory effects are increased beta-adrenoceptor density in rat ventricle after denervation with 6-hydroxydopamine (Yamada et al, 1980) and decreased receptor binding after exposure to isoprenaline (Mickey et al, 1976; Marsh et al, 1980). Among the latter may be included increases in cardiac beta-adrenoceptor density and decreases in alpha-adrenoceptor density in experimental hyperthyroidism (Williams et al, 1977; Ciaraldi and Marinetti, 1977; Williams and Lefkowitz, 1979) with decreases in beta-adrenoceptor binding in hypothyroidism (Stiles and Lefkowitz, 1982). Furthermore, in the hyperthyroid state, changes in the ratio of high to low affinity binding sites of the

beta-adrenoceptor for agonists suggest an enhanced ability of the agonist to stabilise the high affinity state of the receptor, in keeping with an increase in receptor coupling to the adenylcyclase system (Stiles and Lefkowitz, 1981).

The concept of a change in the density or functional characteristics of beta-adrenoceptors on sarcolemmal membranes in ischaemic myocardium as a means of altered adrenergic responsiveness has not been extensively investigated, yet is one theoretical explanation of absence of spontaneous catecholamine release shown in Chapter 3. Furthermore, the concept has the advantage of not requiring the synthesis of new receptors as a prerequisite for modulation (unlikely in acutely ischaemic tissue), since altered binding characteristics, unmasking of latent receptor sites by membrane-active enzymes or variable reductions in functional receptor units may all theoretically lead to heterogeneous catecholamine action within the heart and hence an arrhythmogenic effect.

High specific activity radioligands with high affinity have advantages of allowing assay of small receptor populations at low ligand concentrations hence minimising the problem of non-specific binding (Maguire et al, 1977). (±) [I^{125}]-Iodohydroxybenzylpindolol (I^{125}-IHYP) has been widely used in a variety of tissues employing both saturation and competition binding techniques (Charness et al, 1976; Harden et al, 1976). Its high specific activity (>2,000 Ci/mmol), relatively high affinity (K_D approx 10^{-10} M) and ease of assay in biological tissue confer particular advantages for study in myocardium that outweigh its disadvantages of fairly rapid radiochemical decomposition and

existance only as a racemate. Towards the end of the studies described here, an even more specific radioligand (-) [I^{125})-iodopindolol (I^{125}-IP) was described (Barowsky and Brooker, 1980) and was used to investigate the possibility of direct receptor quantification in vivo.

In vitro receptor quantification from equilibrium binding studies has generally been performed using microsomal membrane preparations, because the use of unfractionated membranes yielded excessive non-specific binding. As a result, up to 95 per cent of the total receptor population was discarded (Baker and Potter, 1980), limiting considerably the biological usefulness of any observed change in receptor activity. Furthermore, serious difficulties may arise in the isolation of subcellular fragments from ischaemic myocardium. The process of ischaemia can itself influence membrane recovery. For these reasons, the first part of this study has investigated the use of I^{125}-IHYP in assay of ventricular beta-adrenoceptors in a 'crude' membrane preparation and identified an unexpected source of error using low ligand concentrations as a consequence of endogenous catecholamines. This may result in important underestimation of receptor numbers at single low-dose ligand concentrations. Secondly, the applicability of I^{125}-IHYP and I^{125}-IP to in vivo receptor mapping in normal and acutely ischaemic myocardium has been explored. Such an approach would avoid some of the difficulties mentioned above and allow assessment of regional differences in receptor function across ischaemic myocardium.

METHODS

In vitro studies

Membrane preparation: Adult male Wistar rats (weight 200 - 350 g) were killed by cervical dislocation and the ventricles minced with scissors and homogenised on ice in 0.25 M sucrose, 5 mM Tris-HCl and mM $MgCl_2$ at pH 7.4 ('homogenate'). The homogenate was filtered through two layers of nylon gauze ('crude cell membranes'), centrifuged at low speed (480 g) for 10 minutes and the pellet consisting mainly of cell debris and fibrous tissue separated. Potassium chloride was added to the supernatant (final concentration 0.5 M) to facilitate dissolution of contractile and other proteins (Baker et al, 1980) and the suspension centrifuged at 48000 g ($4^{o}C$) for ten minutes. The pellet was resuspended in 50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.5 at a protein concentration between 0.5 and 1.5 mg/ml ('cell membrane') or resuspended and recentrifuged twice at 48000 g ($4^{o}C$) for ten minutes ('washed cell membranes'). 'Microsomes' were prepared by recentrifugation of the supernatant from the first 48000 g spin at 140,000 g ($4^{o}C$) for one hour and resuspension in Tris buffer. 'Cell water' was the supernatant from this spin. Protein concentrations above 1 mg/ml were measured by the Biuret method (Mokrasch and McGilvery, 1956) and below this by the method of Lowry et al, (1951). Aliquots of the membrane suspensions (50 µl) were assayed for noradrenaline, adrenaline and dopamine as described in Chapter 2.

Denervation: Cardiac denervation was achieved by four doses of intraperitoneal 6-hydroxydopamine (6-OHDA) as the bromide salt dissolved in one per cent (w/v) ascorbic acid. Two doses of 50 mg/kg were given at 24 hour intervals with two further doses of 100 mg/kg nine and ten days after the first dose. Animals were

sacrificed ten days after the last 6-OHDA injection.

Incubations, separation of bound ligand: For saturation binding, ventricular membranes (300 µl) were incubated in polypropylene tubes for one hour at 37°C with 5-1600 pM I^{125}-IHYP (Radiochemical Centre, Amersham, specific activity 2000 Ci/mmol) in the presence and absence of 200 µM (±) isoprenaline. The total assay volume per tube was 500 µl. Bound and free I^{125}-IHYP was separated by vacuum filtration (30 mmHg) through glass fibre filters (Whatman GF/C) presoaked in 200 µM isoprenaline to minimise non-specific binding to the filter. After washing (3 x 10 ml Tris-buffer), the bound ligand was assayed directly using a gamma counter (NE 1600). Specific binding of I^{125}-IHYP was defined as the amount of radioactivity displaced by 200 µM (±) isoprenaline and varied between 50 and 85 per cent of total binding over the range of ligand concentrations used. At least ten different ligand concentrations were used for each saturation isotherm. For kinetic binding experiments, bound and free ligand were separated at intervals during one hour incubation as described above. For dissociation experiments, bound and free ligand were separated at intervals after the addition of 200 µM (±) isoprenaline to membranes previously incubated for one hour with the radioligand. Total binding to ventricular membranes was kept at less than 10 per cent of the ligand concentration for all studies to avoid errors from the use of excessive receptor concentrations (Chang et al, 1975). I^{125}-IHYP was used within two months of delivery for all experiments.

In vivo studies

I^{125}-IHYP: Adult mongrel dogs were anaesthetised with pentobarbitone and prepared surgically as described in Chapter 3. Thirty minutes after completion of surgery, approximately 100 μCi I^{125}-IHYP was injected intravenously. According to the extent of radiochemical decay, the specific activity of I^{125}-IHYP was reduced 3-5 fold with hydroxybenzylpindolol to achieve a final plasma concentration of approximately 50 pM. At this concentration significant functional beta-adrenoceptor antagonism is unlikely.

Sixty minutes after injection of I^{125}-IHYP (at steady state), the LAD was occluded for thirty minutes (n = 4) and the area of cyanosis and systolic bulging carefully noted. The heart was then excised with the occlusion clip in situ and suspended in a cradle to facilitate coronary perfusion with 500 ml isotonic saline at $4^{o}C$. The saline was infused into the aortic root by roller pump at 100 ml per minute, the occlusion clip being removed immediately prior to the perfusion. Perfusate was collected from the coronary sinus at intervals during the infusion. Epicardial fat and coronary vessels were removed from the left ventricle and the free wall was then cut into a matrix of 80 biopsies including the whole of the ischaemic and border-ischaemic area. Each biopsy was further divided into endocardial and epicardial segments by midline dissection before weighing and direct counting in a gamma counter.

Non-specific binding of I^{125}-IHYP was defined in separate experiments by the relationship between arterial and myocardial concentrations of I^{125}-IHYP in the presence of a saturating amount (1 mg/kg) of propranolol (ICI Pharmaceuticals) given 30 minutes before radioligand injection. Incremental doses of I^{125}-IHYP were injected intravenously and 60 minutes after each increment, a

biopsy of the front wall of the left ventricle was taken (approximately 100 mg), washed, weighed and counted. Simultaneously, arterial plasma was assayed for I^{125}-IHYP. Sixty minutes after the highest dose of I^{125}-IHYP, the heart was excised, perfused and the free left ventricular wall biopsied as described above. In two of the four experiments in which non-specific tissue binding was determined, the LAD was occluded for 30 minutes after the final dose of I^{125}-IHYP. The dose of (-) propranolol was calculated to saturate 99 per cent of the total myocardial beta-adrenoceptor population, in association with at least a 100 fold shift in the isoprenaline dose-response curve.

I^{125}-IP: The ligand was prepared by iodination of (-) pindolol essentially as described by Barowsky and Brooker (1980), originally modified from Maguire et al (1976). Briefly, 10 µl of 13.5 mM HCl containing 20 µg (-) pindolol, 20 µl 0.3 M KH_2PO_4 (pH 7.6), ImCi NaI^{125} (as the carrier-free compound) and 20 µl aqueous solution of chloramine T were combined in that order and the iodination allowed to proceed for 5 minutes at room temperature. The reaction was stopped by addition of 300 µl $Na_2S_2O_5$ (1 mg/ml) in 1M acetic acid. Iodinated products were extracted x4 with 300 µl portions of ethylacetate containing 0.01 per cent phenol. Phase separation was carried out using a Pasteur pipette. The 4 washes were combined, spotted on to a 25 x 40 cm strip of Whatman 3 MM paper and chromatographed for 4 hours at 4°C in 1M ammonium formate (pH 8.5) containing 0.01 per cent phenol. The I^{125}-IP band was located (narrow peak approx. 7 cm from origin), cut into 1 cm strips and the ligand eluted with 5 ml methanol. I^{125}-IP separated well from pindolol. The peak I^{125}-IP bands were combined, evaporated to 1 ml under nitrogen and stored at -20°C.

As preliminary studies in both rat and guinea pig ventricle had suggested that non-specific binding with this ligand was extremely low (< 10 per cent of total binding at 50 - 100 pM) with a Kd of approximately 200 pM, studies were undertaken to determine whether in vivo beta-adrenoceptor binding could be achieved without coronary perfusion as described above and the assumptions implicit in the use of this procedure to remove unbound ligand (see below).

The experimental preparation was the same open-chest pentobarbitone anaesthetised dog described above. Because of the increased affinity of I^{125}-IP for cardiac beta-adrenoceptors, lower concentrations of ligand were injected to avoid functional beta-adrenoceptor antagonism. Coronary occlusion was hence continued for one hour (n = 6) rather than 30 minutes to increase the prospects for steady state bindng in ischaemic tisse, the time to which is directly dependent on ligand concentration. Arterial and coronary venous sampling was continued during the period of ischaemia. In the two control experiments without ischaemia, changes in coronary venous I^{125}-IP were measured during and after supramaximal stimulation of the ansa from the left stellate ganglion. Non-specific binding was measured as described above using 1 mg/kg (-) propranolol given intravenously 30 minutes before radioligand injection. At the end of the period of coronary occlusion the heart was removed, washed ($4^{o}C$) and biopsied directly without coronary perfusion. Biopsies from the ischaemic and non-ischaemic areas were also assayed for NA and A. Tissue for catecholamine assay was immediately frozen on dry ice.

Statistical analysis of changes in adrenoceptor binding to cardiac tissue used analysis of variance for non-ischaemic, border

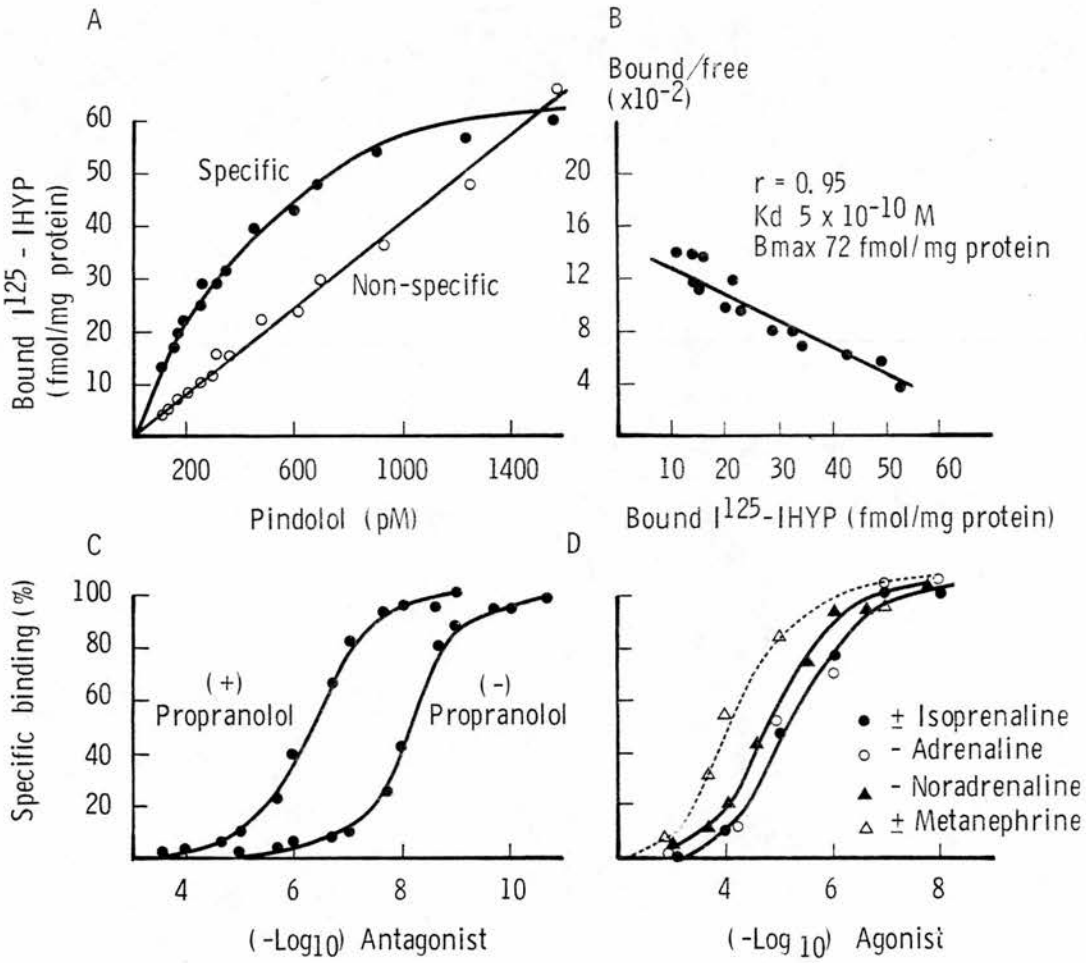


Figure 8.1 Binding of I^{125}-IHYP to rat ventricular membranes: validation studies. Specific binding is saturable (A) and Scatchard analysis (B) suggests a single binding site. Competition for specific binding is stereospecific (C) and conforms to known affinities for a variety of agonists (D). Data from individual experiments, in duplicate. "Cell membrane" preparation.

ischaemic and ischaemic zones, with computed modified t statistic for paired or unpaired samples, as appropriate. A 5 per cent level of confidence was considered statistically significant.

RESULTS

In vitro:

As shown previously (Harden et al, 1976), binding of I^{125}-IHYP to cardiac membranes at concentrations between 100 and 1600 pM was saturable, stereospecific, of high affinity and inhibited by agonists according to their known affinities (Figure 8.1). Scatchard analysis (Scatchard, 1949) suggested a single class of binding sites (Figure 8.1b). Equilibrium was achieved within 60 minutes of incubation at 50 and 200 pM I^{125}-IHYP concentrations (Figure 8.2a) with no tendency for reduction in the free ligand concentration (Figure 8.2b) or change in non-specific binding (Figure 8.2c) over a two hour period under the incubation conditions studied. The 'cell membrane' preparation appeared most suitable for in vitro study as it was the least purified preparation combining the highest specific binding with reasonable non-specific binding at 50 per cent receptor occupancy (Figure 8.3a). Specific binding of pindolol was linear over the range of protein concentration used in this study (Figure 8.3b).

The calculated maximum number of binding sites (Bmax) from six experiments was 61 $\pm$ 7 fmol/mg protein (mean $\pm$ SEM) and affinity (Kd) 460 $\pm$ 70 pM. Kinetic analysis of the association and dissociation of I^{125}-IHYP to the cell membrane preparation also suggested a single binding site. The calculated pseudo first order association rate constant (k_1) was 0.046 pM^{-1} per minute and the

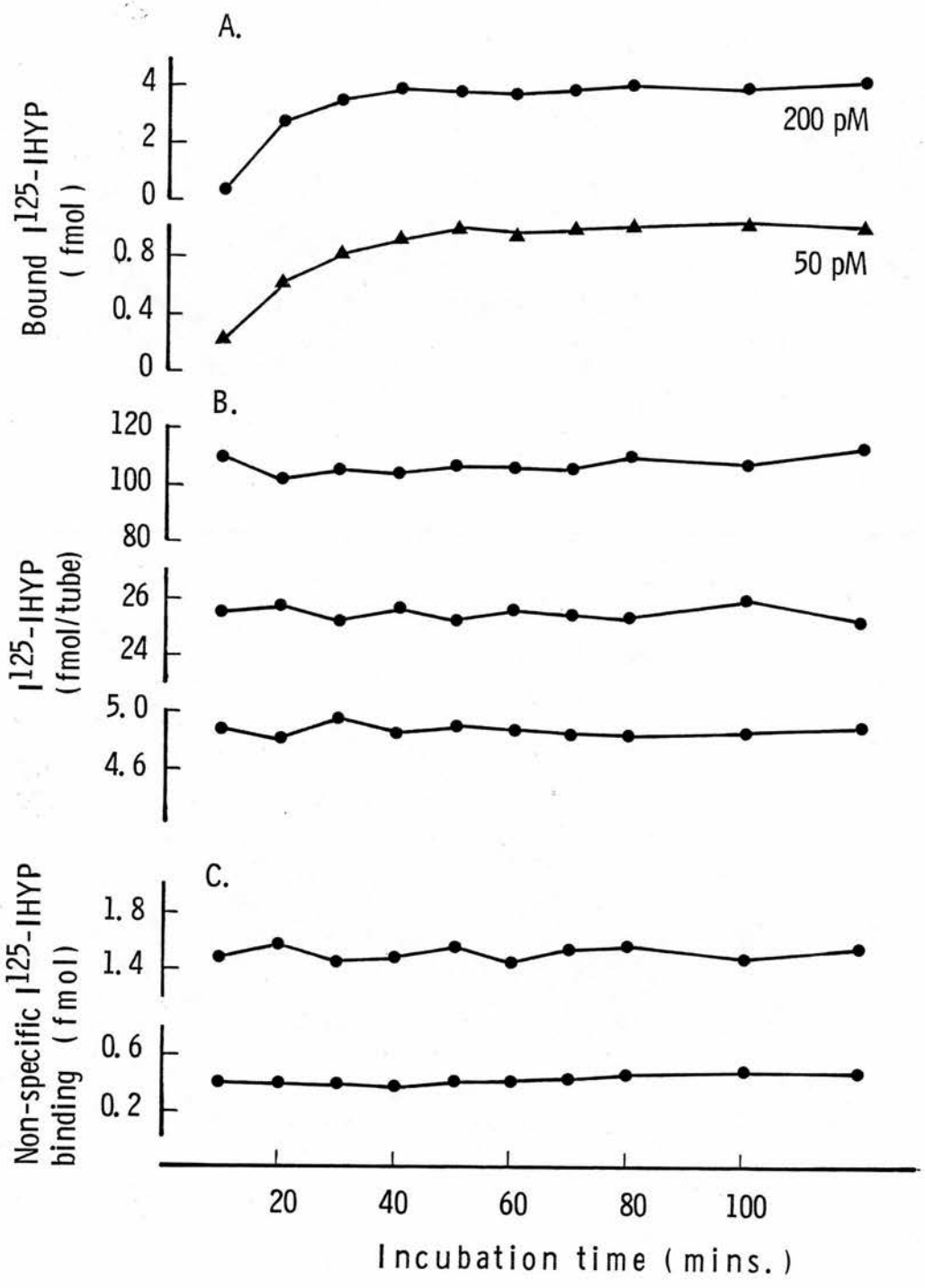


Figure 8.2 Binding of I^{125}-IHYP to rat ventricular membranes. validation studies. Equilibrium is achieved within 60 minutes at 50 and 200 pM I^{125}-IHYP (A). Total radioactivity per tube (B) and binding (C) are independent of incubation time at various concentrations of I^{125}-IHYP. Data from individual experiments in duplicate. "Cell membrane" preparation.

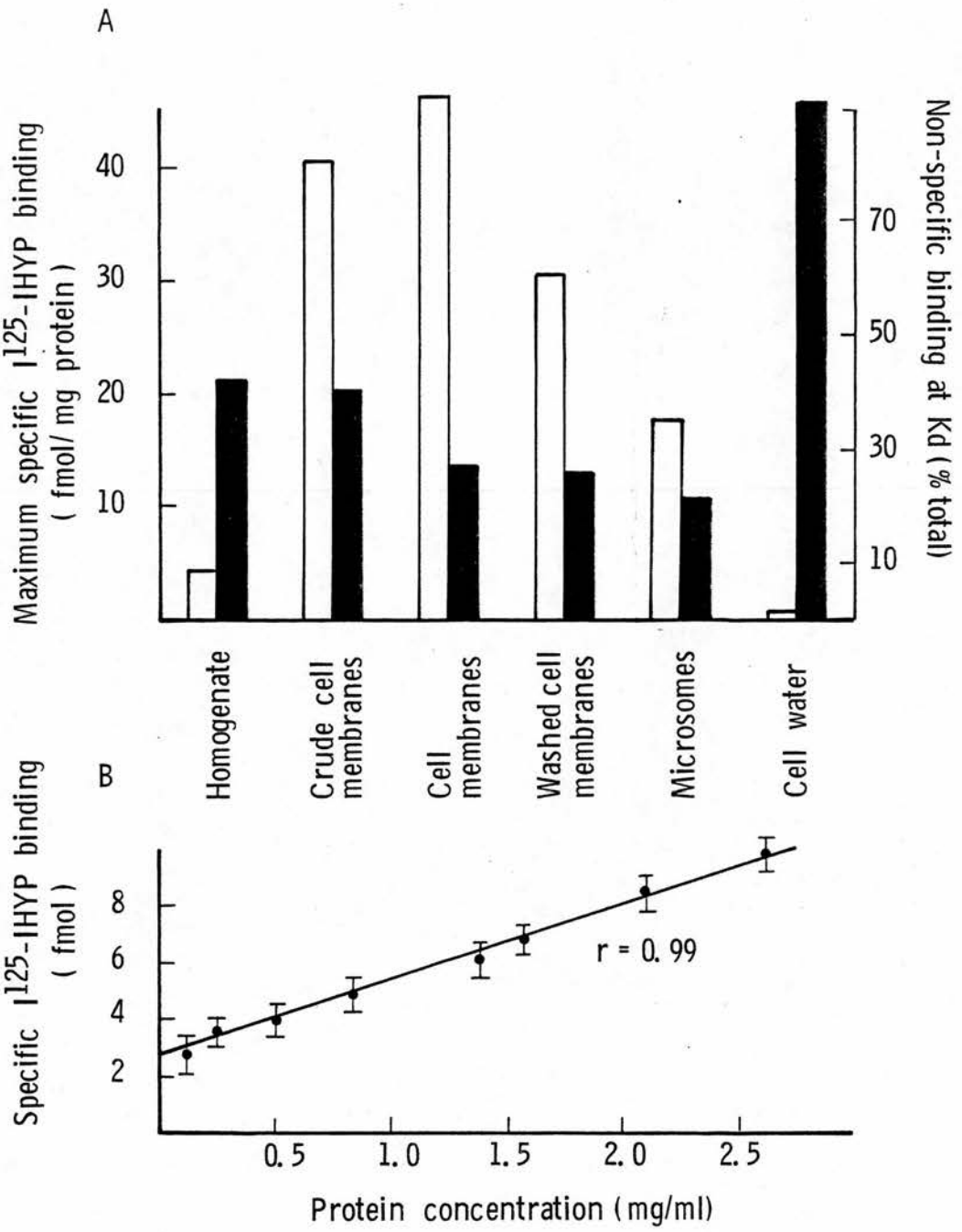


Figure 8. 3 A Specific (open bars) and non-specific (shaded bars) I^{125}-IHYP binding to rat ventricular membranes (see text for definitions). The "cell membrane" preparation combined maximum specific binding with acceptably low non-specific binding. Mean data from two experiments in duplicate. B Linear relationship between specific I^{125}-IHYP binding and measured total protein concentration. "Cell membrane" preparation (mean ± SEM; 3 expts).

second order dissociation rate constant ($k_{obs}-k_1$) 0.021 per minute. The derived Kd from kinetic studies was thus 80 pM, significantly lower than the dissociation constant from equilibrium binding as noted in previous analyses of adrenergic receptors in a variety of tissues (Greenberg and Snyder, 1978; U'Pritchard et al, 1978; Karliner et al, 1979). Mean data from which the association and dissociation rate constants were derived are shown in Figue 8.4.

At lower concentrations of I^{125}-IHYP in the range 5-100 pM, a progressive reduction in expected specific binding to the cell membrane preparation occurred, producing a sigmoid saturation isotherm, an example of which is shown in Figure 8.5. A Hill plot of this data (Hill, 1910) showed a Hill coefficient of 1.4.

Scatchard analysis produced a curvilinear line with upwards convexity (Figure 8.6a) suggesting positive cooperativity between ligand binding sites (Boeynaems and Dumont, 1975) i.e. an increase in drug affinity for the receptor with increasing receptor occupancy. The reduction in specific binding was most readily demonstrated in membranes used within two hours of preparation. Since [NA] containing synaptosomes were expected to be present in the membrane fraction, the hypothesis was tested that this non-linear relationship was due to endogenous catecholamines in the membrane fraction interacting in some way with I^{125}-IHYP for receptor sites. In four experiments, the concentration of I^{125}-IHYP was determined at which specific binding was reduced by 50 per cent from that predicted from the higher part of the saturation isotherm, assuming binding obeyed the law of mass action. This concentration (i50, Figure 8.5) was closely related to the measured endogenous [NA] content of the membrane suspension

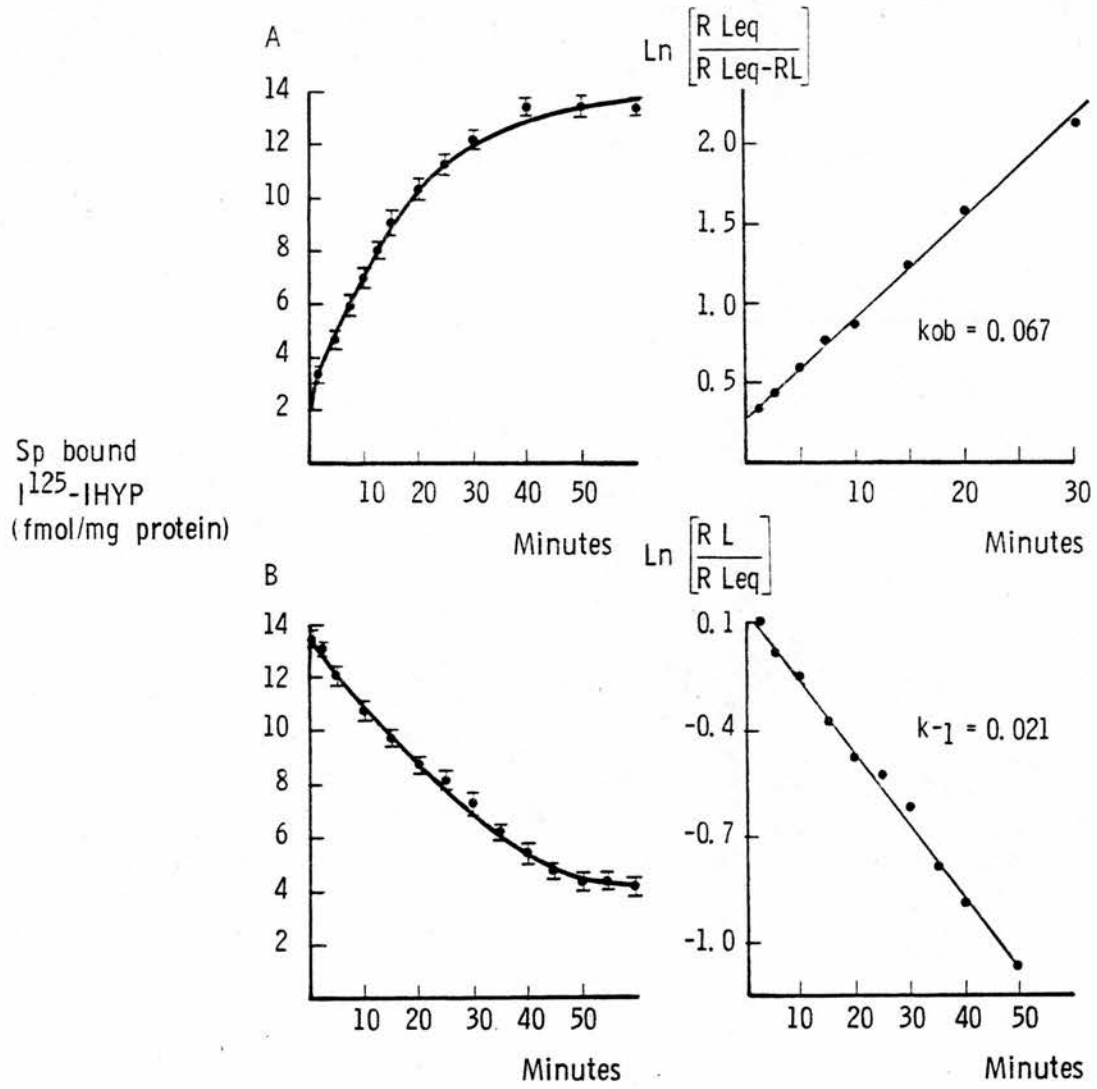


Figure 8.4 Association (A) and dissociation (B) of I^{125}-IHYP from rat ventricular membranes (mean ± SEM, three expts).

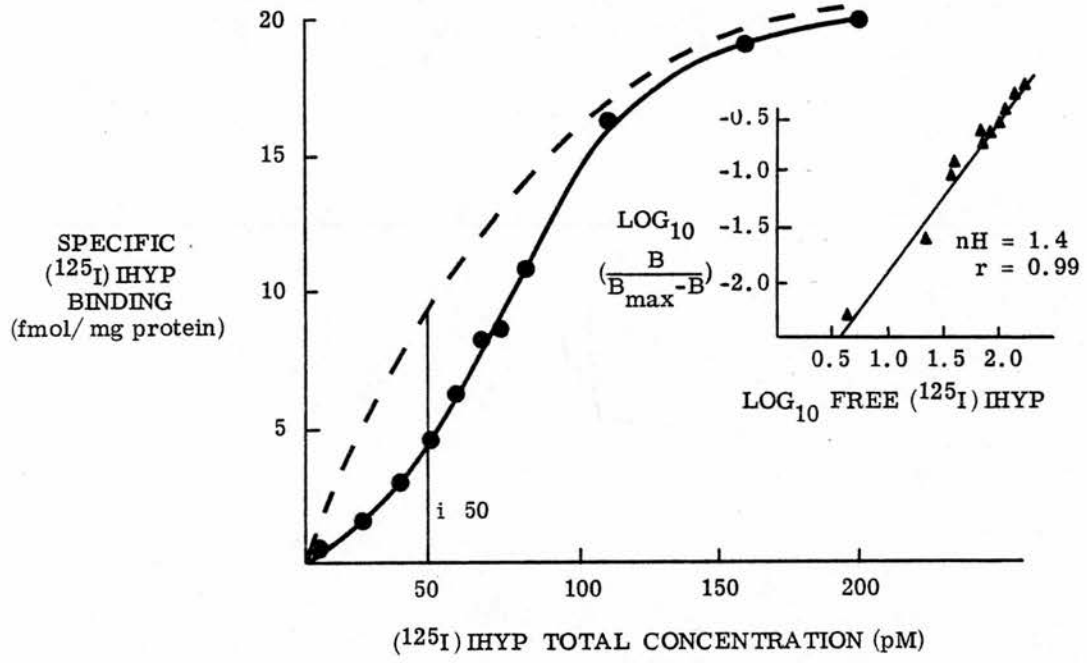


Figure 8.5 Saturation isotherm for I^{125}-IHYP binding in low concentration to rat ventricular cell membranes. The sigmoid saturation curve was representative of four experiments, although the concentration of I^{125}-IHYP at which specific binding was reduced 50 per cent from that predicted from a higher concentration saturation isotherm (the dashed line), the I_{50}, varied with each membrane preparation.

(Figure 8.6b). The [NA] concentration theoretically required to give equivalent membrane binding to I125-IHYP was calculated according to the Cheng and Prusoff equation (Cheng and Prusoff, 1973) using a Kd for (-) NA of 2 μM derived from competition binding experiments with the same membrane preparation. The endogenous [NA] concentration of the membrane suspension was approximately four fold lower than that predicted from the Cheng and Prusoff equation. Concentrations of [A] in the membrane suspension varied between 1 and 2.6 nM and [DA] varied between 2 and 19 nM, both orders of magnitude too low to significantly modify ligand binding.

When endogenous catecholamines were removed in vitro by repeated washing of the 'cell membrane' preparation or in vivo by chemical sympathectomy withn 6-OHDA, a linear Scatchard plot was obtained with no evidence of cooperativity (Figure 8.6a). The calculated Kd (mean $\pm$ SEM) was similar in three experiments between washed (300 $\pm$ 90 pM) and denervated (380 $\pm$ 90 pM) membranes. Bmax, however, increased from 52 $\pm$ 15 to 68 $\pm$ 12 fmol/mg protein with denervation. Membrane [NA] content was substantially reduced in the washed preparation with further reduction after 6-OHDA (Figure 8.7a).

It has been suggested that deterioration of membrane receptor proteins during equilibrium binding might be different in the presence and absence of a non-saturating amount of radioligand producing curvilinear Scatchard analysis. This was investigated by the addition of a saturating concentration of I^{125}-IHYP to washed membranes previously incubated for 120 minutes with 10 pM I^{125}-IHYP. The maximum specific binding obtained after a further

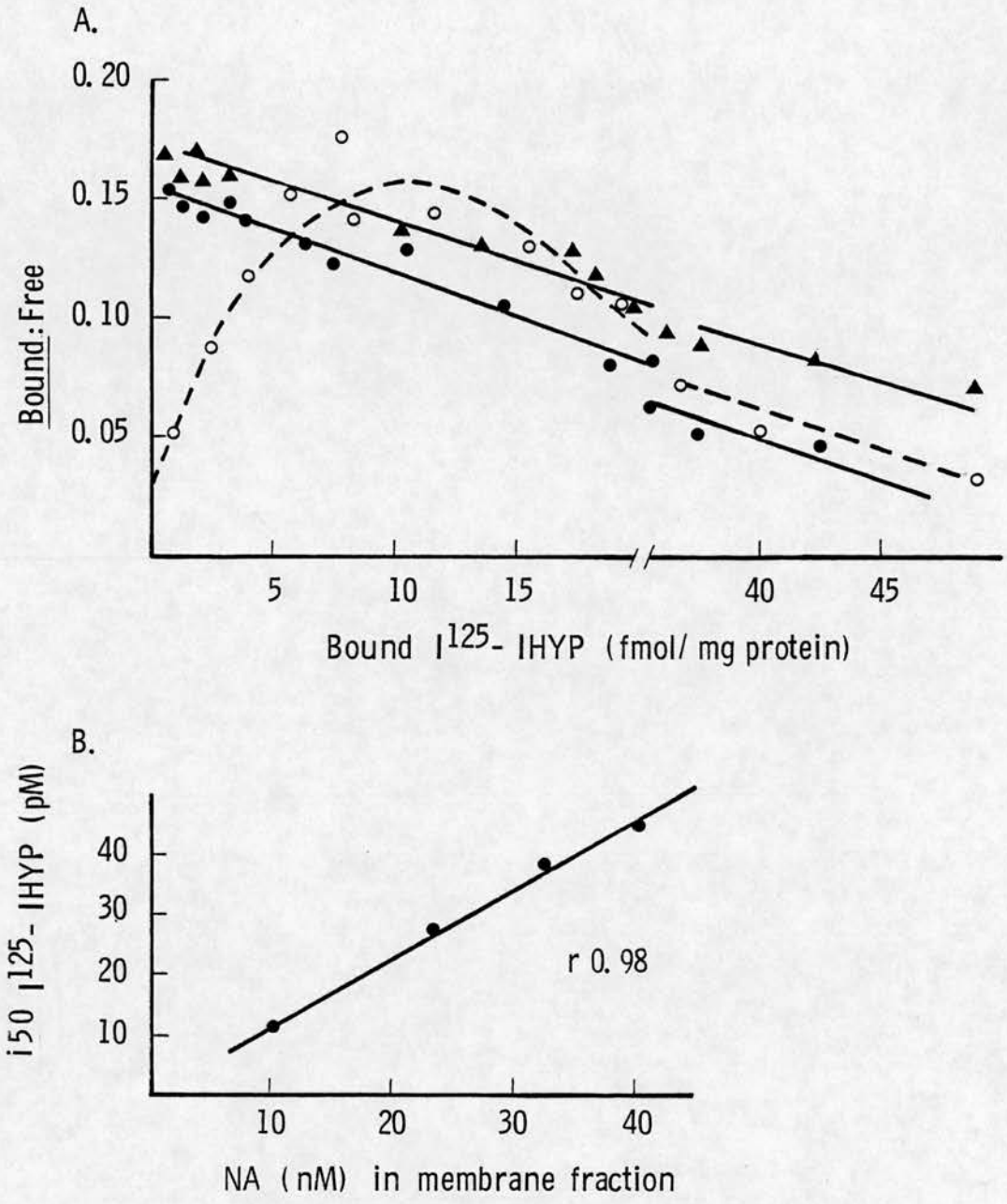


Figure 8.6 A Scatchard analysis of I^{125}-IHYP binding to rat ventricular membranes. "Cell membrane" preparation (open circles); "washed cell membranes" (closed circles); "cell membranes" from denervated animals (triangles). Data from 10 denervated and 10 control (sham-injected) rats. B The relationship between NA content in the cell membrane fraction and I^{125}-IHYP concentration at which specific binding was reduced 50 per cent from that predicted (see figure 8. 5). Data from four experiments.

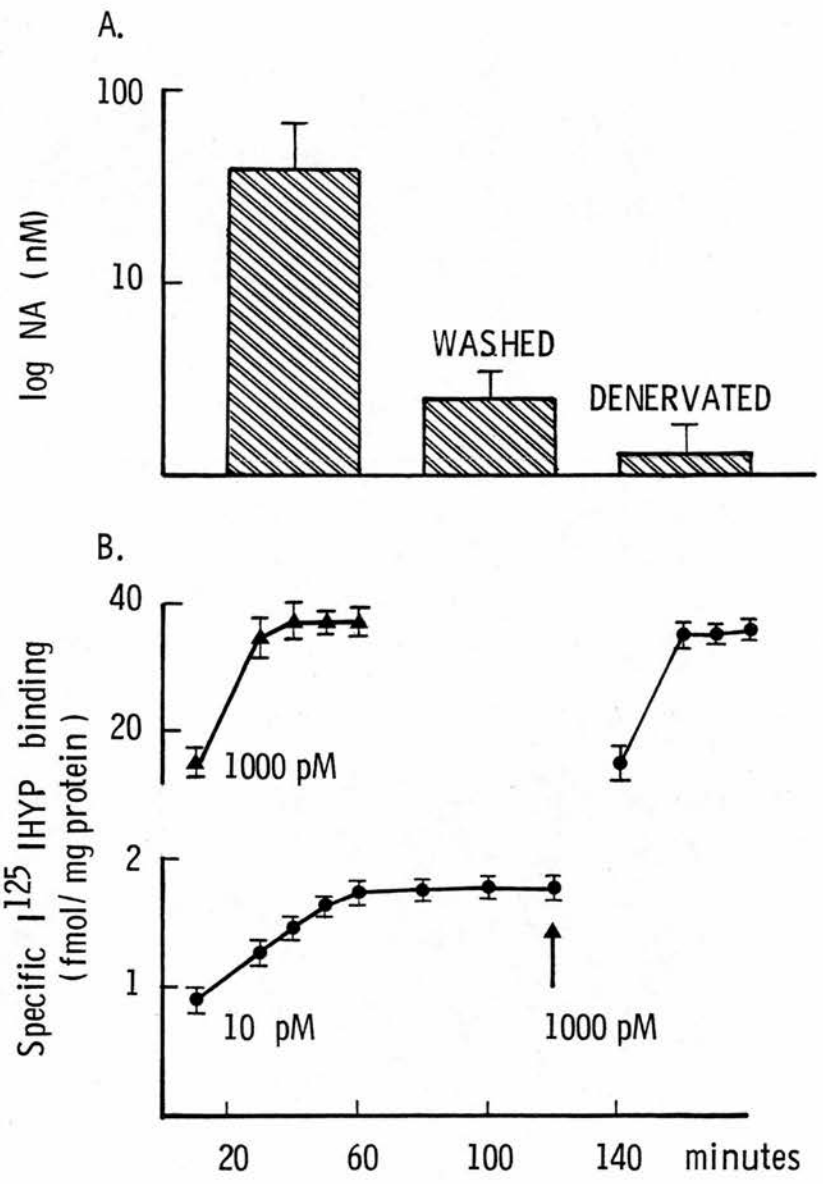


Figure 8.7 A Comparison of endogenous NA content in the three membrane fractions. B Kinetic analysis of I^{125}-IHYP binding to ventricular membranes. After incubation at 10pM for two hours (circles), the addition of a saturating concentration of radioligand (arrow) resulted in similar binding to that obtained with a saturating concentration throughout (triangles). Data mean ± SEM, three experiments.

60 minutes was the same in four experiments as that obtained with a saturating concentration throughout (Figure 8.7b).

In vivo:

I^{125}-IHYP: Non-specific binding of I^{125}-IHYP to ventricular myocardium was linear over a range of ligand concentrations with no evidence of saturability (Figure 8.8a). The slope factor relating arterial I^{125}-IHYP (pM) and non-specific tissue binding to ventricle (fmol/g wet weight) averaged 0.41 over four experiments. No systematic differences between non-specific binding to ischaemic and non-ischaemic areas was observed. Washout of free radioligand during coronary reperfusion was monoexponential and rapid (Figure 8.8b) suggesting removal predominantly from a single compartment.

Changes in specific I^{125}-IHYP binding to ischaemic, non-ischaemic and border ischaemic areas from four experiments are shown in Table 8.1. Considerable interexperimental variability in binding, related principally to variable arterial ligand concentrations, was observed. However, non-specific binding, derived from the arterial I^{125}-IHYP concentration at the end of each experiment and the slope factor from the experiments with (-) propranolol, averaged 47 $\pm$ 6 per cent of total counts. Just over half of the total counts in each biopsy therefore represented binding to the beta adrenoceptor population. No significant changes in adrenoceptor binding were observed in the border ischaemic endo- or epicardial layers. Indeed binding increased in two, decreased in one and was unchanged in the fourth study. However, a small increase in receptor binding (6 $\pm$ 2 per cent) was observed in the central ischaemic endocardium in each experiment,

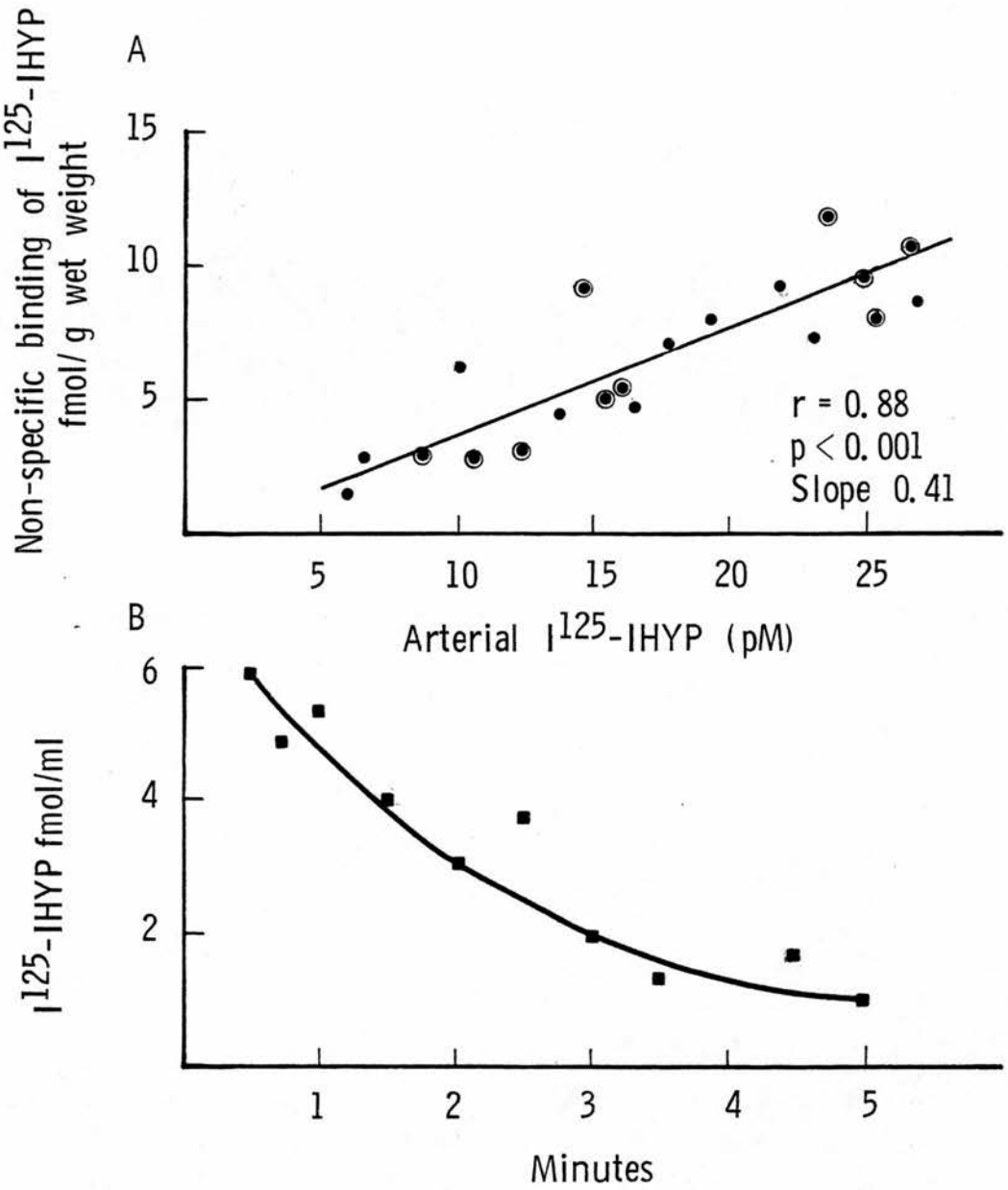


Figure 8.8 A Non-specific radioligand binding to dog myocardium in vivo; relationship to arterial ligand concentration at steady state. Circled points from ischaemic myocardium. Individual data from four experiments. B Effluent I^{125}-IHYP during coronary perfusion with 500ml saline. Monoexponential washout is evident from one experiment after in vivo receptor saturation with l-propranolol.

NON SPECIFIC BINDING (% total)	n	NON ISCHAEMIC Endo	Epi	n	BORDER ISCHAEMIC Endo	Epi	n	CENTRAL ISCHAEMIC Endo	Epi
44	20	12.2 ± 0.3	12.6 ± 0.3	12	11.8 ± 0.3	11.1 ± 0.2	15	12.4 ± 0.5	10.6 ± 0.2
62	24	7.6 ± 0.4	7.1 ± 0.4	7	7.7 ± 0.8	7.1 ± 0.9	11	7.9 ± 0.2	7.0 ± 0.4
35	30	28.6 ± 0.9	29.0 ± 1.0	15	29.1 ± 1.1	27.6 ± 0.9	13	30.3 ± 1.1	28.6 ± 0.9
46	22	24.1 ± 0.8	23.0 ± 0.8	12	25.4 ± 0.9	24.2 ± 1.1	14	27.2 ± 1.0	24.9 ± 0.9
Mean 47 ± 6 SEM	24 ± 6	18.1 ± 4.9	17.9 ± 4.9	12±2	18.5 ± 5.2	17.5 ± 5.0	13±1	19.5 ± 5.5	17.8 ± 5.3

Table 8.1: I^{125}-IHYP binding (fmol/g wet weight) to ischaemic and non-ischaemic dog myocardium in vivo. For each of the four experiments, the per cent contribution of non-specific to total binding is given (derived from figure 8.8A) together with the number of biopsies (n) in each region of interest.

while binding to the corresponding epicardial zone was unchanged. The increase in binding was statistically significant at the 5 per cent level for the second experiment and at the 1 per cent level for the third and fourth experiments. The increase for grouped data however, did not achieve statistical significance at the 5 per cent level. The endocardial : epicardial binding ratio increased in the central ischaemic area in all four experiments from 1.02 $\pm$ 0.02 to 1.11 $\pm$ 0.02 ($p < 0.05$).

An example of the variability in binding across the left ventricle is shown in Figure 8.9. Profiles of endocardial and epicardial binding from one experiment are shown for 50 biopsies including the ischaemic area. Greater variability in binding is evident within the ischaemic region particuarly in endocardium.

I^{125}-IP: The criteria for identification of beta-adrenoceptors were not met by the results using the experimental protocol described above for this series. It is probable that the major reason for this lay in failure to differentiate specific from non-specific binding across the heart. This is illustrated in Figure 8.10 which compares arterial and tissue levels of I^{125}-IP in six experiments using the radioligand alone and in four where beta-adrenoceptors were initially saturated with 1 mg/kg (-) propranolol. It can be seen from this figure that the ratio of plasma to tissue counts was <u>not</u> importantly lower in the propranolol treated group, preventing the assessment of the true receptor population. In keeping with this finding, the concentration of I^{125}-IP in arterial, ischaemic and non-ischaemic coronary venous plasma did not significantly change with systemic injection of a saturating concentration of (-) propranolol nor with

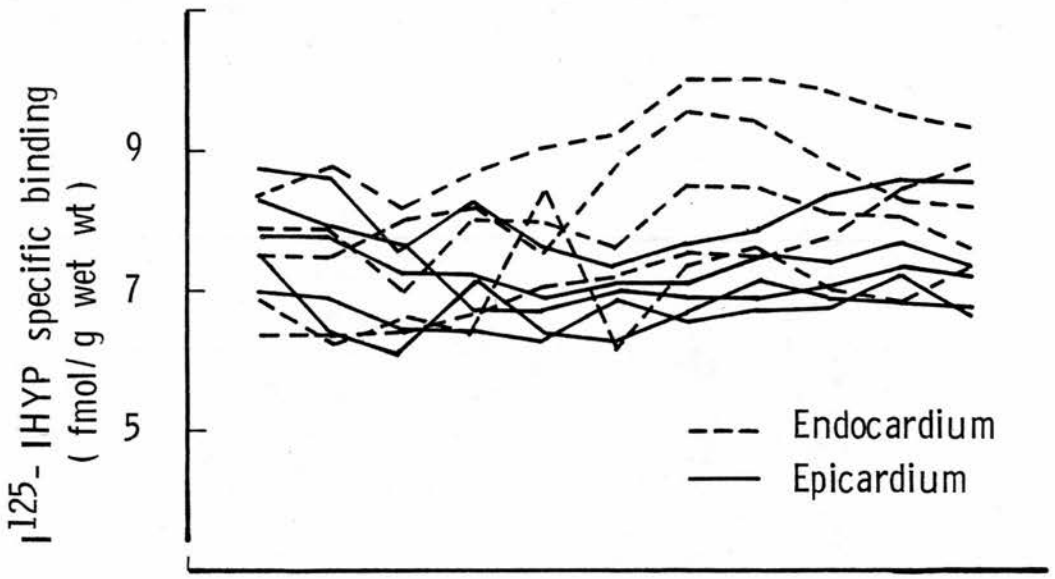


Figure 8.9 Profiles of I^{125}-IHYP binding to canine myocardium in vivo. Data from one experiment shows binding across 5 rows each of 10 sequential biopsies traversing the ischaemic area. Greater variability in binding is seen in the central (ischaemic) area.

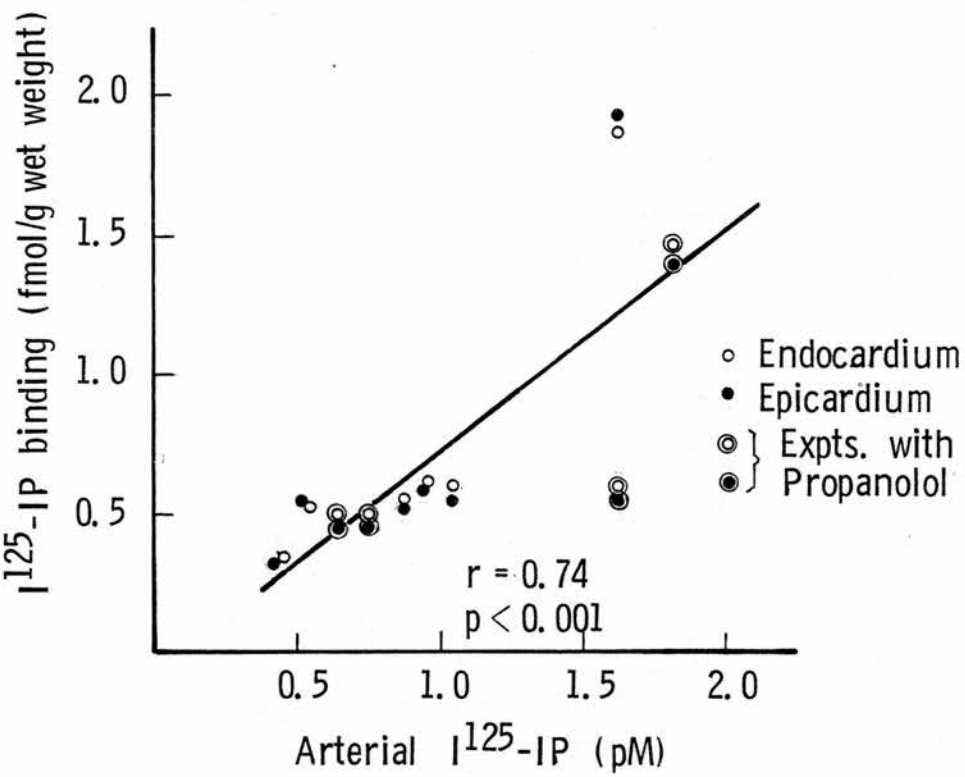


Figure 8.10 Total and non-specific binding of I^{125}-IP to dog myocardium in-vivo; relationship to arterial ligand concentration at steady state. No important reduction in tissue binding is evident in the propranolol experiments (circled) for either endocardium or epicardium.

supramaximal stimulation of the left stellate ganglion (Table 8.2).

The [NA] content of left ventricular muscle was similar from the ischaemic (0.51 ± 0.05 µg/g wet weight) and non-ischaemic (0.51 ± 0.06 µg/g wet weight) regions.

DISCUSSION

In vitro assessment of myocardial beta-adrenoceptors:

This study has demonstrated an important pitfall in the inference of site-site receptor interactions namely that during apparently steady state binding of a high specific activity ligand endogenous tissue hormones can interact with the labelled ligand at the receptor site. Other explanations of the curvilinear Scatchard plot with upwards convexity, including ligand inactivation during incubation, incomplete separation of bound and free forms of the ligand or failure to reach equilibrium (Chamness and McGuire, 1975), seem unlikely because of the relationship between the reduction in specific binding and measured endogenous catecholamines and the demonstration of linear Scatchard analysis after catecholamine depletion both by in vivo and in vitro techniques. This phenomenon may result in significant errors in the estimation of both affinity and receptor density, high affinity, low density receptor populations would not be identified.

It is of interest that the endogenous [NA] concentration of the membrane fraction was lower than that predicted to compete equally with I^{125}-IHYP binding to the membrane receptor population, derived from direct measurement of the affinity of (-) NA under the same experimental conditions. The difference between measured and

	TIME (Minutes)					
	Basal	1	2	4	6	10
ANSA STIM^{n} (20 Hz x 2min)						
A	1940	2000	1950	1930	1950	1890
LV	1820	1790	1760	1810	1440	1600
CS	1880	1890	1810	1880	1720	1890
PROPRANOLOL (1 mg/kg iv)						
A	1820	1800	1810	1790	1780	1780
LV	1570	1580	1600	1560	1550	1560
CS	1560	1570	1590	1550	1550	1540

Table 8.2: Plasma I^{125}-IP (cpm/ml) in arterial and coronary venous plasma before and after supramaximal stimulation of the ansa subclavia from the left stellate ganglion and the systemic injection of a saturating amount of propranolol. Representative data from one of three experiments.

predicted [NA] may relate to non-homogenous distribution of endogenous catecholamines close to receptor binding sites. Thus, the effective [NA] concentration at the receptor site may be higher than the average concentration in the membrane fraction. Alternatively, synaptosome formation in the fraction may have prevented uniform distribution of endogenous neurotransmitter. Equally, the contribution of catecholamine metabolites to ligand displacement has not been quantified.

The discrepancy between the predicted and measured [NA] concentration and the curvilinear nature of the saturation isotherm before removal of endogenous neurotransmitter suggest that, under these incubation conditions, the effect of endogenous catecholamines at low radioligand concentrations does not conform to the law of mass action. One possible explanation for these findings may be that at low concentrations of radioligand, endogenous catecholamines modulate radioligand access to the receptor site and prevent establishment of true equilibrium with the receptor population. Further kinetic studies would be necessary to examine this. A further problem concerns the contribution of the (+) and (-) isomers of I^{125}-IHYP to total binding at low radioligand concentrations. Theoretical considerations predict that a significant proportion of the (+) isomer will bind at low receptor occupancy whereas the (-) isomer (with higher affinity) predominates towards receptor saturation (Burgisser et al, 1981). The effect of endogenous catecholamines with mixed ligand binding is unknown.

False-positive cooperativity may be a more general problem of tissue-ligand systems than that of the rat myocardium-I^{125}-IHYP

system described here. The artefact may be avoided in several ways. Firstly, ligand concentrations sufficient to minimise the endogenous hormone effect (in this case > 100 pM), secondly choice of a ligand with lower affinity for the receptor or thirdly reduction of the endogenous hormone concentration in the membrane fraction (for example by repeated washing) should all prevent curvilinear Scatchard analysis. It is not necessarily correct to assume that endogenous hormone concentrations are negligible, particularly in a crude membrane preparation.

In vivo beta-adrenoceptor quantification in ischaemic myocardium:

Although the possibility of directly assaying beta-adrenoceptor populations in vivo has many theoretical attractions, particularly the avoidance of preparation of sarcolemmal membrane fractions, this study has identified several practical difficulties in the application of radioligand binding techniques with conventional antagonists.

The most important drawback relates to the high proportion of non-specific binding to myocardial tissue and the extracellular space, such that coronary perfusion is required to washout unbound and albumin-bound ligand and allow the identification of the receptor population. It is unlikely that specifically bound ligand will be removed by coronary perfusion because of the latter's short duration (five minutes) and the high affinity of the ligand for the receptor and hence slow rate of dissociation. However, there can be no guarantee that washout from the ischaemic area will be the same as from the non-ischaemic area, particularly since after a thirty minute, and to a greater extent after a sixty minute period of acute ischaemia, the water content of ischaemic tissue will have

increased by up to 30 per cent (Kloner et al, 1974). However, no significant differences in I^{125}-IHYP binding to ischaemic and non-ischaemic myocardium were observed in the propranolol treated animals, suggesting that differential washout of ligand from these two areas would not account for the results.

A second limitation of this in vivo technique is that binding is assessed at only one ligand concentration and not over a range of receptor saturation. Hence, an increase in specific binding may occur either from an increase in receptor density or an increase in receptor affinity for the ligand. Non-specific binding is minimised by the use of low ligand concentrations but too low a concentration prolongs the time required for requilibration of binding following coronary occlusion and may risk errors due to the presence of endogenous neurotransmitter described above. Equilibration of the ligand with the receptor <u>after</u> coronary occlusion would result in reduced ligand availability to the ischaemic receptor pool, or at least in prolongation of the time to equilibration and would preclude its quantitative analysis.

Despite these important limitations, however, the technique for beta adrenoceptor labelling in vivo using I^{125}-IHYP did appear to label a myocardial receptor pool and demonstrated small increases in binding within the receptor pool, associated with a significant increase in endo-epicardial binding ratio, the latter possibly a consequence of more intense ischaemic injury in the subendocardial layers. In addition, variability in ligand binding was enhanced across the ischaemic zone. This technique, however, will require further refinement and in vivo validation before observed changes in receptor binding can be considered to be

relevant to changes in catecholamine sensitivity.

The mechanism or mechanisms responsible for a change in receptor binding within 30 minutes of LAD occlusion are unknown. Theoretically, an increase in receptor binding sites could reflect increased de novo receptor synthesis, translocation of a cytosolic receptor pool to the sarcolemma or unmasking of latent receptors by membrane-active enzymes. In view of the 30 minute time period and the presence of ischaemic injury, de novo synthesis of new membrane receptors seems most unlikely, in contrast to changes in receptor density as a result of thyroid hormone administration (Kempson et al, 1978). Changes in ligand binding to isolated membrane fractions have been described in reticulocyte ghosts incubated with S-adenosylmethionine (Strittmatter et al, 1979) in sarcolemmal membranes treated with detergents or ionophores (Shutt et al, 1977) and in frog erythrocytes treated with phospholipase A_2 (Limbard and Lefkowitz, 1976). Thus, membrane active enzymes may unmask cryptic binding sites, possibly by uncovering receptors in a more fluid membrane environment. This does not necessarily imply enhanced adrenergic responsiveness unless the receptors remain coupled to intracellular readout systems. Part of the desensitization of chick ventricle by exposure to isoprenaline for example, may involve GTP-sensitive reduction in adenylate cyclase responsiveness without change in receptor binding (Marsh et al, 1980).

Natural variations in the density of beta-adrenoceptors across the left ventricle is an unlikely explanation of the findings, since the diaphragmatic surface of the heart and interventricular septum, were not included in the assay. Although beta-adrenoceptor density varies significantly in different chambers of the canine

heart, increasing progressively from atrium to right ventricle, interventricular septum and left ventricle (Baker et al, 1980) and is lower in the diaphragmatic surface of the left ventricle (Burman et al, 1981), no differences within the anterolateral free wall of the left ventricle have been noted (Mukherjee et al, 1979).

Mukherjee et al, (1979) using in vitro binding techniques with (H^3)- dihydroalprenolol noted a significant increase in beta-adrenoceptor density in canine ischaemic myocardium after one hour of LAD occlusion. This increment, however, was maintained over eight hours, beyond the period of irreversible cell injury and at a time when tissue catecholamine depletion had developed, thus raising doubts about the physiological significance of the observation. Binding of a muscarinic cholinergic antagonist, (H^3)-quinuclidynl benzilate, did not change with ischaemia. In a follow-up study, these authors (Mukherjee et al, 1982) noted enhanced catecholamine stimulated cyclic AMP formation and phosphorylase b to a conversion in canine left ventricle after one hour of ischaemia and fifteen minutes reperfusion, providing one biochemical measure of the functional responsiveness of the receptor increase noted earlier. Down regulation of beta-adrenoceptors in non-ischaemic myocardium following regional infarction has also been demonstrated (Baumann et al, 1981) and postulated as an adaptive mechanism to enhanced catecholamine stimulation at this site.

Alteration in alpha-adrenoceptor density in ischaemic and reperfused myocardium has also been described (Corr et al, 1981) and advanced as a possible explanation for enhanced alpha adrenergic activity at these times (Sheridan et al, 1980). In the

study by Corr et al, $alpha_1$-adrenoceptor density , assessed by H^3-prazosin, doubled after 30 minutes ischaemia from LAD occlusion in the anaesthetised cat model, and remained elevated during early (2 minutes) but not late (15 minutes) reperfusion. Na^+-K^+-ATP^{ase} activity remained unaltered during occlusion and reperfusion.

Alterations in cardiac membrane adrenoceptor activity as a result of acute ischaemia requires considerable further evaluation before it can be established as a mechanism for altered sympathetic responsiveness in vivo and before its importance in the genesis of early ventricular arrhythmias in man can be appreciated. Nonetheless, the development of in vivo binding techniques is of critical importance in avoiding possible artefacts due to tissue fractionation and destruction or removal of a functionally important receptor population. The use of ligands with lower affinity for adrenoceptors requires further study for two reasons. Firstly, requilibration as a result of changes in the receptor population will be achieved more rapidly and secondly, changes in ligand distribution or washout in vivo may reflect local release of endogenous neurotransmitter at post-synaptic sites. The development of immunological probes for adrenoceptor binding may be a promising alternative approach (Wrenn and Haber, 1979).

9 SUMMARY AND CONCLUSIONS

The mechanism of ventricular fibrillation in acutely ischaemic myocardium remains poorly understood. Much clinical and experimental evidence suggests a central role for the sympathetic nervous system in the genesis of early ventricular arrhythmias. A combined biochemical, neuropharmacological and electrophysiological approach has been used in the studies reported here to investigate the activity of the sympathetic neuroeffector junction in ischaemic myocardium at the time of onset of early ventricular arrhythmias. A simple animal model has been developed with the aim of increasing understanding of the role of myocardial catecholamines in the development of arrhythmias and their potential relevance to sudden cardiac death in man.

Acute myocardial ischaemia was created by clip occlusion of the left anterior descending coronary artery in the open chest, anaesthetised dog. Coronary venous effluent (draining ischaemic and non-ischaemic myocardium), regional myocardial blood flow (using radiolabelled microspheres) and epicardial activation abnormalities (multielectrode epicardial electrograms) were monitored on a minute-to-minute basis before and during sympathetic stimulation and pharmacological and metabolic manipulation of sympathetic nerve terminals and post synaptic junctions.

Endogenous [NA] concentrations in local venous effluent were not modified by a ten minute period of LAD ligation but myocardial [NA] release was observed to occur from the previously ischaemic area during the first two minutes of coronary reperfusion. It is unlikely that spontaneous [NA] release during ischaemia was masked

by enhanced [NA] metabolism as increased efflux of radioactivity was not observed when the heart was prelabelled by H^3-noradrenaline. Furthermore, failure to sample from the ischaemic area, insensitivity of the preparation to changes in myocardial [NA] release, or the use of a specific anaesthetic agent did not explain absence of spontaneous [NA] release. In occasional individual experiments, sudden increases in [NA] in ischaemic venous effluent were observed coincident with spontaneous ventricular arrhythmias. Spontaneous ventricular fibrillation was associated with marked increases in [NA] in arterial and coronary venous plasma, probably a secondary response to circulatory arrest.

Arterial [A] and, to a lesser extent [NA] rose progressively during coronary occlusion and reperfusion. Extraction of [A] was increased selectively across the ischaemic area during coronary occlusion and was abolished during the first 3-5 minutes of reperfusion, compatible with some release of [A] from the heart at this time.

Thus, as a result of these studies showing absence of sponteneous release of [NA] into coronary venous effluent during acute ischaemia- in contrast with the widely held view- further studies investigated the activity of the sympathetic nerve terminal at varying times after coronary occlusion and its modification by neuronal reuptake and alpha$_2$-adrenoceptor blockade.

The relationship between the duration of ischaemia and responsiveness of cardiac sympathetic nerves has been examined by electrical stimulation of the ansa subclavia from the left stellate

ganglion and measurement of epicardial activation abnormalities and [NA] release from ischaemic and non-ischaemic areas at intervals following LAD ligation. In normal myocardium, sequential one minute periods of ansa stimulation over three hours resulted in reproducible, frequency dependent regional myocardial [NA] release without tachyphylaxis. Two successive ten minute periods of ischaemia did not modify peak myocardial [NA] release from the predominantly ischaemic (I) or non-ischaemic (NI) areas during low (1 Hz) and high (10 Hz) frequency ansa stimulation. Peak [NA] release from the anterior local vein at 1 Hz was 1.1 $\pm$ 0.8 pmol/ml before and 2.2 $\pm$ 1.9 pmol/ml during ischaemia and at 10 Hz was 7.5 $\pm$ 4.3 pmol/ml before and 7.8 $\pm$ 5.0 pmol/ml during ischaemia (p = NS). Reperfusion induced a 30 fold increase in [NA] output (pmol/ml) from I while output from NI was unchanged.

In a second experimental group, myocardial catecholamine release was related to changes in epicardial activation delay during repeated ansa stimulation on four occasions during 75 minutes of ischaemia. Stimulation at the two periods of peak spontaneous phase I arrhythmias 5 and 17 minutes after coronary occlusion resulted in [NA] release from the ischaemic area of 2.8 $\pm$ 1.3 and 3.0 $\pm$ 1.6 pmol/ml respectively and a significant increase in mean activation delay in I of 12 $\pm$ 4 msecs at 5 minutes and 9 $\pm$ 4 msecs at 17 minutes ($p < 0.05$). In contrast, stimulation 30 and 60 minutes after coronary occlusion, when spontaneous arrhythmias are rare, was not associated with [NA] release from I (0.3 $\pm$ 0.3 and 0.9 $\pm$ 0.5 pmol/ml respectively) and resulted in a minor reduction in mean activation delay in I of 2 $\pm$ 3 msecs at 30 minutes and 3 $\pm$ 4 msecs at 60 minutes. [NA] release from NI, and increases in blood pressure and myocardial lactate

release were similar during each of these four periods of ansa stimulation. Coronary reperfusion at 75 minutes induced massive release of [NA] (peak 11.8 $\pm$ 2.8 pmol/ml) and abolished extraction of [A] from I, with a time course similar to the development of the reperfusion arrhythmias which are recognised to occur in this model. Thus, stimulation-evoked release of [NA] from ischaemic myocardium is maintained during the early periods of enhanced vulnerability to arrhythmias but is inhibited after 30 minutes. This temporal variability may be a factor in the time course of spontaneous arrhythmias.

The influence of presynaptic alpha-adrenoceptor and neuronal reuptake blockade, singly and in combination, on basal and nerve stimulated catecholamine responses during coronary occlusion has been investigated using the alpha 2-adrenoceptor antagonist yohimbine and reuptake blockers desmethylimipramine and viloxazine.

Spontaneous [NA] release from I did not occur after yohimbine (1 mg/kg) but release was significantly potentiated during low and high frequency ansa stimulation (at 10 Hz, [NA] release increased from 4.3 $\pm$ 0.4 to 11.8 $\pm$ 5.4 pmol/ml; $p < 0.005$), associated with enhanced release on reperfusion. Arterial [NA] changed little after yohimbine but [A] rose 2-3 fold. Extraction of [A] across the heart was enhanced after yohimbine and enhanced extraction was maintained (across I and NI) during coronary occlusion. Mean arterial blood pressure fell after yohimbine but the increment in blood pressure with ansa stimulation was unaffected. Blood flow to the ischaemic endocardium and epicardium fell significantly by 24

and 36 per cent respectively while flow to the non-ischaemic area was unaffected. Regional epicardial activation abnormalities in I were intensified by yohimbine both before and during ansa stimulation (control 41 $\pm$ 2 msecs, yohimbine 54 $\pm$ 5 msecs at 3 minutes; control 57 $\pm$ 7 msecs, yohimbine 73 $\pm$ 10 msecs during 10 Hz SNS; $p < 0.01$) with in particular an increase in variability of regional activation as assessed by multielectrode grid mapping within the ischaemic area. The incidence of spontaneous ventricular fibrillation increased from 22 to 56 per cent with this drug.

Changes in regional myocardial catecholamine release and blood flow were qualitatively similar after desmethylimipramine (2.5 mg/kg). Spontaneous [NA] release from I or NI was not observed during LAD occlusion, but release during ansa stimulation and reperfusion was substantially increased. Expressed as the increase in [NA] release, potentiation with reuptake blockade following desmethylimipramine was greater across I compared to NI at 1 Hz ansa stimulation and on reperfusion suggesting that there was selective enhancement of reuptake in I prior to drug administration. Despite absence of change in arterial pressure, flow to the ischaemic endocardium and epicardium fell significantly by 44 and 35 per cent respectively, somewhat greater than the fall to the respective non-ischaemic areas. The incidence of spontaneous ventricular fibrillation increased from 17 to 50 per cent.

Given in combination, yohimbine and desmethylimipramine resulted in a 15 fold rise in arterial catecholamines, much greater than that seen with either drug given alone. In the non-ischaemic

heart spontaneous release of [NA] was not seen. During LAD occlusion, however, small but significant spontaneous release of [NA] from I and NI was observed, contrasting with responses to either drug given alone. Increments in coronary venous [NA] (from the higher basal level) with ansa stimulation were modest. [A] extraction across I and NI was maintained with the drug combination and abolished on reperfusion. Epicardial activation abnormalities and their variability were further intensified by the drug combination with a 59 per cent increase in mean activation delay at 3 minutes (from 42 $\pm$ 2 to 67 $\pm$ 6 msecs; $p < 0.01$) and a 55 per cent increase during 10 Hz ansa stimulation (from 56 $\pm$ 5 to 87 $\pm$ 9 msecs; $p < 0.01$). The incidence of spontaneous ventricular fibrillation increased from 14 to 57 per cent.

Myocardial lactate production occurred throughout all periods of ischaemia and remained restricted to I with yohimbine, desmethylimipramine and the combination. Lactate production tended to increase during sympathetic stimulation and with the drug combination was greater than control during sympathetic stimulation and immediately prior to coronary reperfusion.

Selective enhancement of [NA] extraction across I during LAD occlusion (from 42 $\pm$ 6 to 51 $\pm$ 7 per cent; $p < 0.05$) was confirmed in control studies, prior to viloxazine by pulse labelling the myocardium with 1-H^3-noradrenaline. Viloxazine (5 and 25 mg/kg) reduced myocardial extraction of H^3noradrenaline across both I and NI but the percentage reduction was significantly greater across NI (45 $\pm$ 8 per cent) than across I (20 $\pm$ 4 per cent), despite enhanced extraction of [NA] across I before viloxazine. Prelabelling studies with desmethylimipramine showed a

similar trend. The incidence of spontaneous ventricular fibrillation increased from 22 to 44 per cent after viloxazine. In individual experiments, differential reduction in [NA] extraction across I and NI increased the incidence of spontaneous arrhythmias.

These observations on the effects of yohimbine, desmethylimipramine and viloxazine show that presynaptic mechanisms (reuptake and alpha-adrenoceptor negative feedback) are important and are active in controlling [NA] release from acutely ischaemic myocardium at the time of onset of early ventricular arrhythmias. Local neuronal [NA] reuptake is seen to be enhanced in acute ischaemia and this, combined with alpha-adrenoceptor mediated negative feedback limits [NA] release from the heart. Blockade of both processes is necessary to unmask spontaneous release of [NA] from the heart following LAD occlusion. This occurs although the activity of neuronal reuptake blocking drugs, as indicated above may be limited in ischaemic myocardium. While extraction of [A] is enhanced across the ischaemic area, probably secondary to a flow-dependent increase in extraneuronal uptake, extraction of [A] is abolished during early coronary reperfusion. Post-synaptic alpha-adrenoceptor mediated coronary vasoconstriction may limit collateral coronary perfusion to the ischaemic area and is potentiated by $alpha_2$-adrenoceptor antagonism and reuptake blockade. Spontaneous ventricular arrhythmias, and spontaneous and nerve stimulated epicardial activation abnormalities are increased by all the drugs studied, singly and in combination, in parallel with their potentiation of catecholamine output from the heart. Furthermore, heterogeneity of activation delays within the ischaemic area is increased.

The effects of intracoronary infusion (LAD) of potassium and adenosine, both vasoactive and neurogenically active metabolites known to accumulate in ischaemic myocardium, have been assessed by measurement of [NA] release and changes in blood pressure and coronary blood flow during graded sympathetic stimulation. Potassium infusion produced biphasic effects on nerve stimulated regional [NA] release from the heart. At low dose infusions, [NA] release during ansa stimulation was inhibited (peak release at 20 Hz 10.2 $\pm$ 2.6 pmol/ml control and 2.9 $\pm$ 1.8 pmol/ml with 10 mM KCl; $p < 0.01$), while at high dose, release during ansa stimulation was potentiated (peak release at 20 Hz 13.3 $\pm$ 2.7 pmol/ml with 75 mM KCl; $p < 0.05$). Potassium concentrations in the local coronary venous effluent increased from 3.6 to 9.4 mmol/l during the highest infusion rate. [NA] release from and potassium content of the circumflex coronary territory did not change with the LAD infusion. High dose potassium infusion did not increase coronary vascular resistance in the LAD territory but in that territory was associated with a 25 $\pm$ 10 per cent increase in coronary vascular resistance during maximal ansa stimulation.

Intracoronary adenosine caused progressive local coronary vasodilatation and fall in coronary vascular resistance. At high dose (10^{-3} and 10^{-2} M), adenosine potentiated basal release of [NA] from the heart but inhibited release during maximal ansa stimulation. The changes normally seen in local blood flow and coronary vascular resistance during ansa stimulation were not modified by adenosine, despite major alteration in basal coronary vascular tone.

Thus, both these metabolites of myocardial ischaemia are

theoretically capable of modifying neurosympathetic activity in the heart in vivo and may act diversely as metabolic determinants of [NA] release at nerve terminals during acute ischaemia.

Intravenous pindolol (0.45 mg/kg), a beta-adrenoceptor antagonist with partial agonist activity, reduced [NA] release from NI during maximal ansa stimulation but did not modify release from I during a twelve minute period of LAD occlusion and reperfusion. [A] extraction across the heart was unchanged. Following a reduction in heart rate of 32 $\pm$ 6 beats per minute with pindolol, LAD occlusion was associated with reduced lactate production from I, reduction in blood flow to I and NI, an increase in the endo-epicardial blood flow ratio in the central ischaemic area, and a reduction in regional blood flow variance in the border and central ischaemic areas. Epicardial activation abnormalities in I, responses to ansa stimulation and the frequency of spontaneous ventricular fibrillation were also reduced by pindolol. In a separate series in which heart rate was held constant by atrial pacing, pindolol did not modify lactate production, the endo-epicardial blood flow ratio, basal activation delays or spontaneous arrhythmias, compared to a control occlusion. Blood flow variance within the border and central ischaemic areas was, however, reduced.

Thus, the beneficial actions of pindolol on metabolic, haemodynamic and electrophysiological parameters during acute ischaemia depend principally on reduction in heart rate, primarily an anti-ischaemic rather than an anti-arrhythmic effect.

Reductions in regional variability in blood flow may, however, provide additional protection against re-entrant arrhythmias, although the contribution of partial agonist acitivty to this action remains uncertain.

Adrenergic receptor modification has been recognised as an important mechanism of altered sympathetic responsiveness in several disease states, although changes in receptor binding in ischaemia have not been systematically investigated. A high affinity, high specific activity radioligand, I^{125}-Iodohydroxybenzylpindolol (I^{125}-IHYP), was used to assess beta-adrenoceptor populations in rat myocardium in vitro and in dog myocardium in vivo.

During validation studies using a rat heart crude heart membrane preparation, it was found that at low concentrations of I^{125}-IHYP (below 100 pM), variable and unexpected reduction in specific binding of this radioligand ccurred, suggesting positive cooperativity between receptor sites. The reduction in specific binding paralleled the competition from catecholamines predicted from direct measurement of the concentrations of these in the membrane fraction. Following depletion of endogenous catecholamines by in vivo and in vitro techniques, a simple receptor population of uniform affinity was demonstrated. At low ligand concentrations, therefore, endogenous hormones may interfere with receptor analysis and suggest false receptor site interactions.

In vivo beta-adrenoceptor labelling with tracer doses of

I^{125}-IHYP, subsequent washout of free ligand by coronary perfusion and separate determination of non-specific binding after receptor saturation with l-propranolol was validated and applied to assess regional changes in radioligand binding after 30 minutes coronary occlusion in the open chest anaesthetised dog. High non-specific binding and uncontrolled ligand washout limited considerably the sensitivity of the technique. Nonetheless, small increases in specific I^{125}-IHYP binding to endocardium from the central ischaemic area were observed, with greater variability in ligand binding at this site. Unfortunately, despite promising in vitro results with (-) I^{125}-Iodopindolol (I^{125}-IP), an isomer with, apparently, less non-specific binding than I^{125}-IHYP, in vivo studies with I^{125}-IP did not allow direct labelling of the heart with this ligand. The possible reasons for this and potential applicability of the technique to define receptor and neurotransmitter activity in the heart have been discussed.

These studies indicate the important role of catecholamines in acute myocardial ischaemia at the time of spontaneous ventricular arrhythmias. The release of these catecholamines is determined by complex interactions at sympathetic nerve terminals. The neuro-effector junction is modified by changes in neuronal reuptake, local feedback mechanisms, release of ischaemic metabolites from the myocardium, changes in regional coronary blood flow and post synaptic adrenoceptor activity. Difficult although these studies are, models of this type show promise of gradually elucidating the multifactorial determinants of ventricular fibrillation during acute myocardial ischaemia, a prerequisite for the rational application of therapeutic measures to prevent sudden cardiac death in man.

10 PUBLICATIONS AND PRESENTATIONS

Parts of the material and concepts presented in this thesis have been published, submitted for publication, or presented to learned societies as follows:

(1) **Forfar, J.C.**, Riemersma, R.A., Oliver, M.F. Are myocardial catecholamines released during acute ischaemia? Scottish Medical Journal **26**: 281, 1981. (Scottish Society for Experimental Medicine).

(2) **Forfar, J.C.**, Riemersma, R.A., Oliver, M.F. Sympathetic nerve stimulation and noradrenaline release from acutely ischaemic myocardium. Clinical Science **61**: 3P, 1981. (Medical Reserch Society).

(3) **Forfar, J.C.**, Riemersma, R.A. Quantitative analysis of myocardial adrenergic receptors: false positive cooperative interactions secondary to endogenous catecholamines. In: Advances in Studies on Heart Metabolism. Eds: Caldarera, C.M., Harris, P., Clueb, Bologna, pp. 115-119, 1982. (International Society for Heart Research, 1981).

(4) **Forfar, J.C.**, Riemersma, R.A., Oliver, M.F. Myocardial noradrenaline response to acute ischaemia. British Heart Journal **45**: 350-1, 1981. (British Cardiac Society).

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